ENDOCRINOLOGY

The Bulletin of
The Association for the Study
of Internal Secretions

PUBLICATION BOARD

R. G. HOSKINS, *Editor-in-Chief*

HANS LISSER — FRANCIS M. POTTENGER
OSCAR RIDDLE — EDGAR ALLEN
DAVID P. BARR — MILTON O. LEE

COLLABORATORS

FULLER ALBRIGHT
WAYNE J. ATWELL
GEOFFREY BOURNE
ISRAEL BRAM
JOHN C. BURCH
JOHN C. DONALDSON
MAURICE H. FRIEDMAN
MURRAY B. GORDON
ARTHUR GROLLMAN
FRANK A. HARTMAN
E. C. HAMBLEN
F. C. KOCH
EDWARD LARSON
J. T. LEWIS
JOSEPH M. LOONEY
WARREN O. NELSON
J. P. PRATT
A. T. RASMUSSEN
ELMER L. SEVRINGHAUS
L. B. SHIPNER
D. L. THOMSON
R. L. ZWEMER

VOLUME 21
1937

Medicine owes no thanks to him who, without proof, would formulate her theories.—T. R. Elliott

Copyright, 1937, by The Association for the Study of Internal Secretions, Inc. All rights reserved

Composed, Printed and Bound by
The Collegiate Press
George Banta Publishing Company
Menasha, Wisconsin

medical lit
Wahr

CONTENTS OF VOLUME 21

NO. 1, JANUARY, 1937

Effects of Chronic Implantation of Rats with Pituitaries of the Same Species. *Philip A. Katzman, Nelson J. Wade and Edward A. Doisy* 1
The Friedman Test and Pituitary Tumor. *E. Perry McCullagh and W. Kenneth Cuyler* .. 8
The Site of Formation of the Posterior Lobe Hormones. *Charles Fisher* 19
Experimental Studies of the Anterior Pituitary. IV. The Replacement Capacity and the Non-cyclic Behavior of Homoplastic Anterior Pituitary Grafts. *M. Schweizer, H. A. Charipper and H. O. Haterius* 30
The Importance of Corticoadrenal Regulation of Potassium Metabolism. *R. L. Zwemer and R. Truszkowski* 40
Increased Calcium Appetite of Parathyroidectomized Rats. *Curt P. Richter and John Fulton Eckert* 50
Further Studies on the Action of Parathyroid Extract in the Dog Following Total and Partial Ablation of the Kidneys. *W. R. Tweedy, R. D. Templeton and F. A. McJunkin* 55
Excretion of Male Hormones I. *Charles D. Kochakian* 60
Effects on Spermatogenesis of a Follicle-Stimulating Extract Obtained from Menopausal or Castrate Urines. *John Huberman, Howard H. Israeloff and Benjamin Hymowitz* 67
The Effect of Sex Hormones on Blood-Calcium and Inorganic Blood-Phosphate Levels. *Hubert Whatley Marlow and Fred C. Koch* 72
The Effect on Serum-Calcium and Inorganic Phosphate of Fractions Obtained from Crude Ovarian Extracts. *S. Louise Huey and H. W. Marlow* 85
The Quantitative Determination of the Gonadotropic Material of Urine of Women after Castration and the Menopause and of Normal Men. *Philip A. Katzman* 89
The Effect of Anterior Pituitary-like Hormone on the Blood Picture in Rabbits. *David Wilson* 96
The Phospholipids of the Brain, Kidneys and Heart of White Rats in Experimental Hyperthyroidism. *Arthur Weil* 101
Failure of the Reid Hunt Acetonitrile Reaction as a Clinical Test for Hyperthyroidism. *Roberto F. Escamilla* 109
Clinical and Laboratory Notes
Report of 1600 Aschheim-Zondek Tests (Friedman Modification). *Morris Feresten* 116
The Pressor Effect of Prolonged Administration of Glycerin Extract of Adrenal Cortex. *R. G. Hoskins and J. H. Pierman* 119
Delayed and Incomplete Union of the Epiphyses of the Long Bones. *E. E. Beard* .. 121
Excretion of Gonadotropic Substance in Polycythemia Vera. *S. Leon Israel and Theodore H. Mendell* 123
Current Endocrine Literature 125

NO. 2, MARCH, 1937

Studies on Adaptation. *Hans Selye* 169
Statistical Survey of Endocrinopathies Among Young Delinquents. *Nelson M. Taylor and Robert L. Schaefer* 189
Observations on 'Prediabetes.' *Hugo R. Rony* 195
The Effect of Cortin on the Sodium, Potassium, Chloride, Inorganic Phosphorous and Total Nitrogen Balance in Normal Subjects and in Patients with Addison's Disease. *George W. Thorn, Helen R. Garbutt, Fred A. Hitchcock and Frank A. Hartman* .. 202
The Effect of Cortin upon the Renal Excretion of Sodium, Potassium, Chloride, Inorganic Phosphorous and Total Nitrogen in Normal Subjects and in Patients with Addison's Disease. *George W. Thorn, Helen R. Garbutt, Fred A. Hitchcock and Frank A. Hartman* 213
The Treatment of Undescended Testes with Anterior Pituitary-like Substance. *W. O. Thompson, A. D. Bevan, N. J. Heckel, E. R. McCarthy, and P. K. Thompson* 220
Evaluation of Hormone Therapy for Undescended Testes in Man. *Arthur J. Cramer, Jr.* 230
The Type and Degree of Gonadal Stimulation Induced in Hypophysectomized Male Rats Parabiotically Joined with Castrated, Cryptorchid, and Normal Partners. *Eugene Cutuly, D. Roy McCullagh and Elizabeth Cutuly* 241
The Interrelationship of the Pituitary Sex Hormones in Ovulation, Corpus Luteum

Formation, and Corpus Luteum Secretion in the Hypophysectomized Rabbit. *M. A. Foster, Ruth Caldwell Foster and F. L. Hisaw* 249
Some Factors Influencing the Vitalization of the Ovarian Graft and the Production of Sex Hormones in the Male Rat. *Carroll A. Pfeiffer* 260
Production of Estrogenic Hormone by a Transplantable Ovarian Carcinoma. *Leonell C. Strong, W. U. Gardner and R. T. Hill* 268
The Effects of Thyroidectomy, Castration, Anterior Lobe Administration and Pregnancy upon Experimental Diabetes Insipidus in the Cat. *W. R. Ingram and Charles Fisher* 273
The Effect of Adrenalectomy and Thyroidectomy on Ketonuria and Liver Fat Content of the Albino Rat Following Injections of Anterior Pituitary Extract. *Edith G. Fry* .. 283
Clinical and Laboratory Notes
A Lipoidemia Following Abortion. *Eldon M. Boyd* 292
Pituitary Response to Cervical Dilatation. *Charles H. Birnberg* 294
Association Notice 296
Current Endocrine Literature 297

NO. 3, MAY, 1937

Some Effects of Synthetically Prepared Male Hormone (Androsterone) in the Rat. *Carl R. Moore and Dorothy Price* 313
Changes in the Relative Amounts of the Follicle Stimulating and Luteinizing Hormones in the Hypophysis of the Female Rat under Varying Experimental Conditions. *Samuel L. Leonard* 330
The Action of Pituitrin, Estradiol and Progesterone on the Human Uterus in Vivo. *Raphael Kurzrok, Hans Wiesbader, Michael G. Mulinos and Benjamin P. Watson* . 335
Comparative Action of Gonad-Stimulating Hormones on the Ovaries of Rats. *H. L. Fevold, F. L. Hisaw and R. O. Greep* 343
The Effect of Complete and Partial Hypophysectomy in Adult Albino Rats on Water, Chloride, Sodium, Potassium and Sulfur Metabolism. *Marta Sandberg, David Perla and Olive M. Holly* 346
The Metabolism of Water, Chloride, Potassium, Sodium, Calcium, Magnesium and Phosphorus in Suprarenalectomized Rats. *Marta Sandberg, David Perla and Olive M. Holly* 352
Observations on Carbohydrate Metabolism Following Irradiation of the Pituitary Gland. *M. Pijoan and Robert Zollinger* 357
The Effect of Female Sex Hormones upon the Oxygen Consumption Rate of Normal Rats, and upon the Tolerance to Desiccated Thyroid. *D. N. Danforth, R. R. Greene and A. C. Ivy* 361
Experimental Acute Hyperparathyroidism. 1. Chemical Studies. *A Cantarow, J. T. Brundage and E. L. Housel* 368
Blood Chemistry of Surviving Parathyroidectomized Dogs. *Everett I. Evans, S. Szurek and R. Kern* 374
Carbohydrate Metabolism Studies in Hypophysectomized Rats. *Leo T. Samuels and Howard A. Ball* 380
Case Reports
A Study of the Metabolism of a Dwarf under Treatment with Growth Hormone. *H. C. Struck and S. A. Szurek* 387
Transplantation of Adrenal Tissue in Addison's Disease. *Max A. Goldzieher and S. B. Barishaw* 394
Tuberculosis of Suprarenal Glands (Addison's Disease). *Henry M. Feinblatt and Barnett Alpert* 401
Pubertas Praecox (Macrogenitosomia). *Homer P. Rush, J. B. Bilderback, Donald Slocum and Arthur Rogers* 404
Clinical and Laboratory Notes
A Short Test for Ovarian Follicular Hormone and Other Estrogens. *Edgar Allen, George M. Smith and W. U. Gardner* 412
Estrogenic Hormones in the Ovaries of Swordfish. *Abner I. Weisman, Daniel I. Mishkind, Israel S. Kleiner and Christopher W. Coates* 413
Association Notice 415
Current Endocrine Literature 417

NO. 4, JULY, 1937

Neural and Endocrine Factors in Bodily Defense. *F. M. Pottenger* 449
Emphasis of the Growth Effect of Prolactin on the Crop Gland of the Pigeon by Arrest of Mitoses with Colchicine. *C. P. Leblond and Edgar Allen* 455
The Effects of Hypophyseal Implants from Normal Mature Guinea Pigs on the Sex Organs of Immature Guinea Pigs. *Ida Genther Schmidt* 461

The Effects of Hypophyseal Implants from Guinea Pigs with Irradiated Ovaries on the Sex Organs of Immature Guinea Pigs. *Ida Genther Schmidt* 469
Effect of Emmenin on Gonadotropic Hormone Excretion in Castrates and Spontaneous Menopause. *Udall J. Salmon and Robert T. Frank* 476
The Effect of Hypophyseal Injection and Implants on the Activity of Hypophysectomized Rats. *Curt P. Richter and John F. Eckert* 481
The Excretion of Gonadotropin by Normal Human Males after the Ingestion and Injection of Extracts of Pregnancy Urine. *Maurice H. Friedman and George Louis Weinstein* 489
Ovaries Secrete Male Hormone. *R. T. Hill* 495
Male Hormone and the Testis-Comb Relationship in the Chick. *W. R. Breneman* 503
Reversal of Estrin-Induced Prostatic Pathology in Mice by the Use of Testosterone. *Harold P. Rusch* 511
The Use of Glycerol Extract of the Adrenal Cortex in the Treatment of Adrenal Insufficiency. *Frank A. Hartman, George W. Thorn and Rollin R. Durant* 516
Factors Determining and Limiting the Growth of Transplanted Suprarenal Cortical Tissue. *Leland C. Wyman and Caroline tum Suden* 523
Evidence of the Protective Influence of Adrenal Hormones Against Tuberculosis in Guinea Pigs. *F. M. Pottenger, Jr. and J. E. Pottenger* 529
The Specificity of Progesterone in Inducing Sexual Receptivity in the Ovariectomized Guinea Pig. *Roy Hertz, R. K. Meyer and M. A. Spielman* 533
The Effect of Artificially Raised Metabolic Rate on the Electro-Encephalogram of Schizophrenic Patients. *Morton A. Rubin, Louis H. Cohen and Hudson Hoagland* .. 536
The Influence of Fasting on Thyroparathyroidectomized Albino Rats. *R. D. Templeton, E. L. Borkon, Valeria Genitis and E. A. Galapeaux* 541
Case Reports
Clinical Experiences with Protamine Insulin in Its Various Modifications. *Abraham Rudy* 544
Fatal Functional Uterine Bleeding. *E. C. Hamblen and D. H. Sprunt* 553
Cachexia Hypophyseopriva (Simmonds' Disease). *Robert P. Regester and Tracy D. Cuttle* 558
Current Endocrine Literature 561

NO. 5, SEPTEMBER, 1937

The Functional Efficiency of Transplanted Adrenal Cortical Tissue. *Leland C. Wyman and Caroline tum Suden* 587
The Use of Estrin in the Treatment of Eclampsia. *Evan Shute* 594
Complete and Incomplete Estrogenic Hormones, Arising from Different Sites in the Rat's Ovary. *S. C. Freed and Samuel Soskin* 599
Comparative Action of Testosterone Compounds, of Estrone and of Combinations of Testosterone Compounds and Estrone on the Anterior Hypophysis. *J. M. Wolfe and J. B. Hamilton* 603
The Spermatogenic and Secretory Function of the Gonads of Hypophysectomized Adult Rats Treated with Pituitary FSH and LH. *Roy O. Greep and Harry L. Fevold* 611
The Quantitative Assay of "Follicle Stimulating" Substances. *Louis Levin and H. H. Tyndale* 619
Epithelial Growth Caused by Stimulation with Various Smear Methods as Demonstrated by Mitotic Stasis with Colchicine. *Philip V. Rogers and Edgar Allen* 629
Ovaries Secrete Male Hormone. III. Temperature Control of Male Hormone Output by Grafted Ovaries. *R. T. Hill* 633
Acceleration of the Rate of Passage of Fertilized Ova Through the Fallopian Tubes of Mice by Massive Injections of an Estrogenic Substance. *H. O. Burdick and Rae Whitney* 637
Differential Diagnosis of Pseudocryptorchidism and True Cryptorchidism. *James B. Hamilton and Gilbert Hubert* 644
Treatment of Sexual Underdevelopment with Synthetic Male Hormone Substance. *James B. Hamilton* 649
Testis Hormone in Relation to Age. *Charles W. Hooker* 655
The Biological Effects of Thymectomy. *Nathan H. Einhorn and Leonard G. Rowntree* .. 659
Studies on the Physiology of Lactation. VII. Lactation in Thyroidectomized Rats and Guinea Pigs. *Warren O. Nelson and Charles E. Tobin* 670
A Comparison of the Ketosis Developed During Fasting by Obese Patients and Normal Subjects. *Eaton M. McKay and James W. Sherrill* 677
Clinical and Laboratory Notes
Studies on the Function of the Pineal Gland. *Nelson J. Wade* 681
Study of the Gilfillen-Gregg Skin Test for Pregnancy. *Saim Saglik and Elemer Scipiades, Jr.* 684
Some Observations of the Gonadotropic Hormones of Pregnancy. *Benjamin Tenney, Jr., and Frederic Parker, Jr.* 687

The Bitterling Fish Response to Male Sex Hormones. *Seward E. Owen* 689
The Occurrence of Estrogenic Substances in the Ovaries of Echinoderms. *J. Kenneth Donahue and Earl DeWitt Jennings* 690
Current Endocrine Literature 692

NO. 6, NOVEMBER, 1937

Studies on Corpus Luteum Function. I. The Urinary Excretion of Sodium Pregnanediol Glucuronidate in the Human Menstrual Cycle. *Eleanor Hill Venning and J. S. L. Browne* 711
Responses of the Human Ovary to Gonadotropic Principles. *E. C. Hamblen and R. A. Ross* 722
Some Effects of Estrogens on the Uterus of the Mouse. *W. U. Gardner and Edgar Allen* 727
On the Composite Nature of the Estrus Phenomenon. *S. C. Freed, S. D. Mesirow and Samuel Soskin* 731
The Effect of Graded Doses of Estrin Upon the Pituitary, Adrenal, and Thymus Weights of Mature Ovariectomized Rats. *Henry Lauson, Carl G. Heller and Elmer L. Sevringhaus* 735
The Excretion of Androgenic and Estrogenic Substances in the Urine of Children. *Ralph I. Dorfman, William Walter Greulich, and Charles I. Solomon* 741
Induction of Penile Erection by Male Hormone Substances. *James B. Hamilton* 744
Testosterone and Testosterone Acetate and the Protein and Energy Metabolism of Castrate Dogs. *Charles D. Kochakian* 750
The Effect of Testosterone, Testosterone Propionate and Dehydroandrosterone on the Secretion of the Gonadotropic Complex as Evidenced in Parabiotic Rats. *Roy Hertz and Roland K. Meyer* 756
Plasma Electrolyte Disturbance in Patient with Hypercorticoadrenal Syndrome Contrasted with That Found in Addison's Disease. *Irvine McQuarrie, R. M. Johnson and M. R. Ziegler* 762
Creatinuria and Creatine Tolerance in Childhood. *E. Kost Shelton and B. N. Tager* ... 773
The Effect of Thyroglobulin and Related Substances on the O_2 Consumption of Liver in Vitro. *Attilio Canzanelli and David Rapport* 779
Effects of Thyroidectomy and Thyroid Feeding in Geese on the Basal Metabolism at Different Temperatures. *Milton Lee and Robert C. Lee* 790
The Effect of Hypoglycemia on the Metabolism of the Brain. *H. E. Himwich and J. F. Fazekas* 800
The Effect of Splenectomy on the Weight of the Hypophysis of the Albino Rat. *Linden F. Edwards and Clarence W. Wright* 808
Alterations in the Percentage of Cell Types in the Hypophysis by Gonad Transplantation in the Rat. *Carroll A. Pfeiffer* 812
Current Endocrine Literature 821

Errata, Volume 21

On *page 223, last paragraph,* it was erroneously stated that gynantrin (G. D. Searle & Co.) is an anterior pituitary-like substance extracted from pregnancy urine. Gynantrin (Searle) is an extract, containing A. P. sex factors, made directly from fresh anterior pituitary gland.

On *page 389, 7th line from top,* the height of the patient should have been stated as 53 inches instead of 64.5 inches.

ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 21 JANUARY, 1937 NUMBER 1

EFFECTS OF CHRONIC IMPLANTATION OF RATS WITH PITUITARIES OF THE SAME SPECIES[1]

PHILIP A. KATZMAN, NELSON J. WADE AND EDWARD A. DOISY
From the Laboratories of Biology and of Biological Chemistry,
St. Louis University School of Medicine
ST. LOUIS, MISSOURI

Since the work dealing with the loss of sensitivity to hypophyseal extracts exhibited by animals after chronic administration has been reviewed recently by others we shall not attempt to cite all of the publications concerned with this subject.

The cause of the production of this insensitivity is at present controversial. In the main, two explanations have been advanced. The one, postulated by Collip (1), that the refractoriness is a normal reaction in which antihormones hold in balance the activity of the internal secretions, is supported by the following evidence. First, the function of the gonads and thyroids of animals which have become insensitive to gonadotropic and thyrotropic extracts of anterior lobes of a different species is depressed below normal, indicating perhaps a neutralization of the animal's own pituitary hormone (2, 3, 4, 5, 6, 7, 8). In line with this is the evidence that the serum of a horse which had received chronic treatment with thyrotropic extract was capable of reducing the basal metabolic rate of normal rats (2). Second, thyrotropic and gonadotropic inhibitory substances have been reported to be present in the blood of untreated people and animals (9, 10, 11, 12, 13), and finally, Selye et al (7) have found that immature rats receiving chronic treatment with implants of pituitaries from rats had very small ovaries.

The other explanation ascribes the production of insensitivity to the formation of antibodies. The evidence in favor of this view is: first, in nearly all investigations in which insensitivity was induced the experimental animals were injected with extracts prepared from the anterior lobes of a different species. Second, a serum which is inhibitory to an extract of the hypophysis of one species apparently is not inhibitory to an extract of another species (7, 8, 14). Third, if the excess of hormone is supplied by the hypophyses of animals of the same species the refractory state is not obtained (15, 16, 17, 18). Fourth, sub-stimulating doses of heterozoic extracts and inactivated extracts induce insensitivity (18, 19, 20) and finally, it has been

[1] The data presented in this paper were reported at the 1936 Meeting of the American Society of Biological Chemists: (Katzman, P. A., N. J. Wade and E. A. Doisy, J. Biol. Chem. 114: lvi. 1936).

demonstrated that an extract prepared from the pituitary of a heterozoic animal may not induce insensitivity. (21).

It seems to us that the validity of the antihormone theory depends, to a large extent at least, upon the demonstration of the production of insensitivity in animals receiving chronic treatment with pituitary hormones of homozoic animals. Smith (15), Witschi (16), Martins (17), and Twombly (18) have reported negative findings while Selye et al (7) claim to have produced refractoriness by such treatment. In view of these apparently conflicting results we decided to obtain additional data on the effects of chronic implantation of female rats with pituitaries from the same species.

EXPERIMENTAL

Starting at 55 days of age 15 female rats were injected daily with 1 or 2 rats' hypophyses. Donors were of various ages and both sexes but for each day animals of the same sex and age were used, making the treatment uniform for the entire experimental group. After removing the posterior lobe, the anterior lobe was injected as rapidly as possible to minimize any post-mortem changes which might affect the hormone. Some 5000 rats were used to supply the hypophyses for this experiment.

Five of the animals died from ear disease or undetermined causes during the first 2 months of the experiment. Autopsy showed a moderate increase in the size of the ovaries of the animals which died during the first month and a pronounced increase in the second month. The remaining 10 animals survived until the termination of the experiment which, in the case of 2, extended over a period of 9 months. All of these animals were in good health at the time they were sacrificed.

Vaginal smears which were made daily showed that continuous estrus did not occur. After 2½ to 4 months of treatment estrus ceased in all cases but later reappeared within 40 to 110 days in spite of the continued administration of implants. However, the cycles were very irregular with only occasional cornification and in half of the animals estrus did not recur after 1 or 2 cycles. In those instances in which laparotomies were performed during diestrus as well as at the time the cycles reappeared, the ovaries were enlarged. All animals were in the diestrus phase at the time they were sacrificed.

At intervals between 7 and 9 months of treatment 2 of the animals were sacrificed for each test. After decapitation under light chloroform anaesthesia, the blood was collected in the presence of oxalate, centrifuged and the plasma used for the determination of inhibitory substances. The ovaries, adrenals, thyroids and pituitaries were carefully dissected from adhering tissue and weighed.

As can be seen from table 1 the ovaries of only 2 of the 10 animals were within the normal weight range. This does not necessarily imply that these ovaries had regressed since a laparotomy performed on 1 of these animals 4 months after the treatment was instituted revealed little enlargement. In another group of 6 animals started at the same age and similarly treated with 1 pituitary daily for 1 to 2 months, only 1 animal had enlarged ovaries. The

ovaries of the other 5 were of normal weight. Histological examination of these ovaries showed no signs of degeneration.

The ovaries of the other 8 rats of table 1 were from 50 to 400% larger than ovaries of untreated animals. Histological examination revealed that this enlargement was mainly due to the large number of corpora lutea. From these data it is quite evident that the ovaries were not subnormal in weight even after 275 days of treatment with implants. The weights of the other endocrine glands did not differ significantly from those of our normal stock animals.

The plasma was tested for inhibition of gonadotropic extracts by determining whether it would diminish the effects of implantation of rats' anterior lobes. Twenty-one day old female rats were used. Each animal received a total of 2.4 to 3.0 cc. of plasma administered on the mornings and evenings of 3 successive days. This treatment was started 1 day before the administration of implants was begun. Pituitaries for implantation in every case were obtained

TABLE 1. EFFECT OF PITUITARY IMPLANTS ON ADULT FEMALE RATS.

Implantation Period days	Body wt. at Autopsy gms.	Wt. of Ovaries mg.	Wt. of Adrenals mg.	Wt. of Thyroids mg.	Wt. of Pituitary mg.
210	205	145	57.0	13.3	8.7
210	245	180	57.0	15.7	11.0
230	269	90	45.0	17.5	9.5
230	280	218	52.0	15.5	11.5
240	255	200	63.0	16.3	10.7
240	277	120	29.8	17.4	7.5
250	260	96	45.0	19.0	9.7
250	262	62	39.5	16.2	8.4
275	272	56	44.0	20.5	12.0
275	304	130	47.0	24.5	10.0
Average	263	130	48.0	17.6	9.9

from 80 to 100 gram male rats, 40 to 50 days of age. Two implants were given daily for 3 days. Controls received plasma only, implants only or normal plasma plus implants in the same amounts that were given to the experimental animals. The implants as well as the plasma were administered subcutaneously. The animals were killed 100 hours after the first implant was made. The results are shown in table 2.

It is evident that the plasma of the rats which had received chronic treatment did not inhibit the ovarian enlargement induced by hypophyseal implants. In fact, in most of the cases this plasma augmented the response to the implants. The average weight of the ovaries of the immature animals treated in this manner was nearly twice as great as that of animals receiving implants only. The plasma alone did not increase the size of the ovaries. Normal plasma from adult female rats plus implants in amounts given above produced no appreciable augmentation.

It does not appear likely that this augmentation is an effect of the plasma proteins or other inert material in decreasing the rate of absorption of the hormones, as has been described by Maxwell (22), Saunders and Cole (23) and Hellbaum (24), since in our experiments the materials were administered

separately and, furthermore, plasma of normal rats did not exhibit this effect. It seems more probable that this augmentation is due to the presence of some active principle, probably the luteinizing hormone, in the plasma of the experimental animals. The plasma alone had no detectable effect on the immature ovary which would indicate an absence of the F.S.H. Since the ovaries in which augmentation occurred were more highly luteinized than those of the controls which received implants only or implants plus normal plasma, there is some evidence for the belief that the experimental plasma contained luteinizing hormone. Furthermore, the increase in weight of the ovaries of the animals receiving the chronic treatment, as judged by histological examination, was due to an extensive luteinization (differing from the parabiosis experiments) which may have been caused by an excess of the luteinizing hormone.

No evidence was obtained for the existence of thyrotropic inhibitory sub-

TABLE 2. INFLUENCE OF PLASMA ON THE EFFECT PRODUCED BY PITUITARY IMPLANTS IN IMMATURE FEMALE RATS.

Treatment	No. of Rats	Body Weight at Autopsy gm.			Weight of Ovaries mg.		
		Min.	Max.	Av.	Min.	Max.	Av.
Implants only	12	40	48	43	16.0	40.5	28.2
Normal plasma+ implants	6	42	48	45	15.5	43.0	31.9
Experimental plasma only	3	49	52	50	11.8	14.2	13.3
Experimental plasma +implants	10	40	52	44	23.0	83.0	49.0

stance in the plasma of the rats subjected to chronic hypophyseal implants. To determine the presence of such an inhibitory substance 4 to 6-day old guinea pigs were used. Each control (6 animals) received during 3 days the anterior lobes from 6 male rats, 40 to 50 days of age and weighing 80 to 100 gms. Since our supply of plasma was limited, due to the fact that we had not contemplated studying this phase of the problem, only 2 experimental animals were used. Each received a total of 4.0 cc. of plasma given in 6 injections over a 3-day period and in addition 6 hypophyseal implants exactly as administered to the controls. The injection of plasma was begun 1 day before the first implant was made. All animals were autopsied 96 hours after the administration of implants was begun. Histological examination of the thyroids revealed a marked stimulation in both experimental and control guinea pigs (+++ reaction). The possibility that the foreign material of the plasma might have caused hypertrophy of the thyroid and thus have masked the effect of any inhibitory substance was ruled out since plasma alone of normal or hypophysectomized female rats failed to produced any stimulation whatsoever.

DISCUSSION

The data which we have obtained are in agreement with those of Smith (15), Du Shane et al (16), Martins (17) and Twombly (18), in demonstrating that refractoriness does not develop in animals treated with hormones of homozoic animals. These data, however, are not exactly comparable since different methods have been used. Smith implanted the pituitaries of rats into hypophysectomized rats, while in the experiments conducted by Witschi's group and Martins the excess hormone was supplied by the pituitaries of castrate rats in parabiosis with normal or hypophysectomized females. Twombly was unable to detect any inhibitory substance in the blood of women treated with extracts prepared from the urine of pregnant women. The fact that loss of sensitivity does not develop regardless of the mode of administration, adds weight to the evidence that inhibitory substances are not formed in response to an excess of hormone obtained from the same species.

At the present it is difficult to harmonize our data with the report of Selye, Collip and Thomson (7) that rats treated with implants of rats' hypophyses from the 21st day of life for 68 days had very small ovaries. The animals used by us were 55 days of age, approximately the age at which rats of our colony become sexually mature. Likewise the animals used by Witchi's group were post-puberal. Perhaps a partial explanation of this difference of results may be found in the report of Hisaw, Hertz and Fevold (25) that juvenile monkeys develop a refractory state more readily than adults after treatment with a gonadotropic extract of the pituitaries of a different species. It is possible that the difference in the reactions exhibited by juvenile and adult monkeys to heterozoic hormones may occur in rats subjected to an excess of homozoic hormones.

Although we do not doubt that substances are produced which neutralize anterior lobe hormones prepared from heterozoic species it seems to us that these observations can be explained better by the production of antibodies rather than antihormones. Recent publications which have appeared since the completion of the present work support this contention. Werner (21) has found that thyrotropic extracts of beef pituitaries prepared by the Na_2SO_4 method of Van Dyke and Wallen-Lawrence (26) readily induce insensitivity in guinea pigs, whereas flavianate preparations (Meyer's modification of the procedure of Evans, Meyer and Simpson, 27) of equal potency injected over a much longer period produced refractoriness in only a small percentage of the animals. He also found (20) that the injection of non-stimulating doses of the Na_2SO_4 extract rendered the guinea pigs insensitive to larger quantities of the extract. Similar results were obtained by Hertz and Hisaw (19) with non-stimulating quantities of gonadotropic extracts.

Twombly (18) found that the gonadotropic material of urine of pregnancy which was completely inactivated by ageing or almost completely inactivated by boiling readily produced protective substances in rabbits. Furthermore, he was unable to demonstrate the presence of inhibitory substances in the blood of a woman treated with large quantities of the active material for more than a year. It would seem, therefore, that the development of insen-

sitivity depends upon the nature of the extracts used and not the potency.

In a consideration of the suppression of the function of the gonads and thyroids to subnormal levels in animals which have become insensitive to gonad and thyroid stimulating extracts, one should bear in mind the possible influence, direct and indirect, exerted by the internal secretion of the stimulated gland. For example, Loeb (28) believes that the loss of reactivity to thyroid stimulating extract is, in part at least, due to the lowered reactivity of the cells of the thyroid brought about by the increased production of thyroid hormone. It is also possible that the excessive amount of thyroid hormone depresses the thyroid-stimulating activity of the hypophysis (29, 30, 31, 32, 33). This is supported by the finding of Anderson and Collip (2) that the pituitaries of rats resistant to thyrotropic extract are devoid of thyroid stimulating activity.

The depressing action of estrogenic hormones on ovarian function is well known. On this basis it is possible that the subnormal size of ovaries of rats resistant to gonadotropic extracts is due to the excessive amounts of follicular hormone secreted during the period of stimulation. In other words the subsequent decrease in size of the stimulated organ to the subnormal level in animals receiving chronic treatment of heterozoic hormones may be due to: *A*), the formation of neutralizing substances; and *B*), the suppression of hypophyseal activity to a subnormal level.

Furthermore, in work of this type the possible influence of cytotoxins should not be overlooked. The blood of animals treated for prolonged periods with hypophyseal preparations may contain pituitary cytotoxins. This may account for the observation made by Anderson and Collip (2) that the basal metabolism of normal rats is lowered by the administration of serum of a horse which had been treated with thyrotropic extract. However, one is still at a loss to explain the inhibitory action of blood of untreated people and animals reported by Collip and Anderson (9), Eitel and Loeser (10, 11), Scowen and Spence (12) and Laroche and Simonnet (13).

As stated before, the experiments of Smith, Witchi, Martins and Twombly, together with the data presented by us point to the conclusion that resistance to an excess of hypophyseal hormone does not develop if the hormone is supplied by homozoic animals. These findings and observations reported by others indicate that inhibitory substances are formed when the injected extracts are antigenically foreign for the species receiving the extracts and are not necessarily formed when the extracts are not antigenically foreign. However, at the present time it is not possible to correlate all of the available data on the basis of any one theory. It is not unlikely that there may be more than one mechanism for the production of substances which neutralize the hypophyseal hormones of heterozoic animals.

SUMMARY

Female rats receiving chronic implantation of anterior lobes of the same species for as long as 9 months still had enlarged ovaries. No evidence was obtained for the existence of gonadotropic or thyrotropic inhibitory substances

in the plasma of these rats. On the contrary, this plasma augmented the gonadotropic response of immature female rats to implanted anterior lobes. The weights of the adrenals, thyroids, and hypophyses of the animals receiving the chronic treatment did not differ significantly from those of normal rats.

REFERENCES

1. Collip, J. B.: Ann. Int. Med. **8:** 10. 1934.
2. Anderson, E. M., and J. B. Collip: J. Physiol. **82:** 11. 1934.
3. Lee, M. O., and J. Gagnon: Endocrinology **14:** 233. 1930.
4. Korenchevsky, V.: Biochem. J. **24:** 383. 1930.
5. Szarka, A.: In Monograph by H. M. Evans et al. "The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis." University of California Press: Mem. U. Calif. **11:** 369. 1933.
6. Selye, H., J. B. Collip, and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **31:** 487. 1934.
7. Selye, H., J. B. Collip, and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **31:** 566. 1934.
8. Fluhmann, C. F.: Proc. Soc. Exper. Biol. & Med. **32:** 1595. 1935.
9. Collip, J. B., and E. M. Anderson: J.A.M.A. **104:** 965 1935.
10. Eitel, H., and A. Loeser: Klin. Wchnschr. **13:** 1677. 1934.
11. Eitel, H., and A. Loeser: Klin. Wchnschr. **13:** 1742. 1934.
12. Scowen, E. F., and A. W. Spence: J. Physiol. **86:** 109. 1936.
13. Laroche, G., and H. Simonnet: Compt. rend. Soc. de biol. **121:** 416. 1936.
14. Meyer, R. K., and E. L. Gustus: Science **81:** 208. 1935.
15. Smith, P. E.: J.A.M.A. **88:** 158. 1927.
16. DuShane, G. P., W. T. Levine, C. A. Pfeiffer, and E. Witschi: Proc. Soc. Exper. Biol. & Med. **33:** 339. 1935.
17. Martins, T.: Compt. rend. Soc. de biol. **119:** 753. 1935.
18. Twombly, G. H.: Endocrinology **20:** 311. 1936.
19. Hertz, R., and F. L. Hisaw: Am. J. Physiol. **108:** 1. 1934.
20. Werner, S. C.: Proc. Soc. Exper. Biol. & Med. **34:** 392. 1936.
21. Werner, S. C.: Proc. Soc. Exper. Biol. & Med. **34:** 390. 1936.
22. Maxwell, L. C.: Am. J. Physiol. **110:** 458. 1934.
23. Saunders, F. J., and H. H. Cole: Proc. Soc. Exper. Biol. & Med. **33:** 505. 1936.
24. Hellbaum, A. A.: Proc. Soc. Exper. Biol. & Med. **33:** 568. 1936.
25. Hisaw, F. L., R. Hertz, and H. L. Fevold: Endocrinology **20:** 40. 1936.
26. Van Dyke, H. B., and Z. Wallen-Lawrence: J. Pharmacol. & Exper. Therap. **40:** 413. 1930.
27. Evans, H. M., K. Meyer and M. C. Simpson: In Monograph, "The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis." University of California Press: Mem. U. Calif. **11:** 27. 1933.
28. Loeb, L.: Science **80:** 252. 1934.
29. Kuschinsky, G.: Arch. f. exper. Path. u. Pharmakol: **170:** 510. 1933.
30. Hohlweg, W., and K. Junkmann: Arch. f. d. ges. Physiol. **232:** 148. 1933.
31. Aron, M., C. Van Caulaert, and J. Stahl: Compt. rend. Soc. de biol. **107:** 64. 1931.
32. Chen, G., and H. B. Van Dyke: Proc. Soc. Exper. Biol. & Med. **32:** 484. 1934.
33. Marine, D., S. H. Rosen, and C. Spark: Proc. Soc. Exper. Biol. & Med. **32:** 803. 1935.

THE FRIEDMAN TEST AND PITUITARY TUMOR

E. PERRY McCULLAGH AND W. KENNETH CUYLER
Cleveland Clinic
CLEVELAND, OHIO

Excessive quantities of urinary prolan have been reported not only in cases of pregnancy (1, 2, 3), but also in the presence of such conditions as malignant chorionepitheliomata (4), hydatid mole (5), or in tumors which contain chorionic tissue, such as teratomata (6). Hirsch-Hoffman (7) reported 10 cases of pituitary disease, including unverified cases of tumor, in which the Aschheim-Zondek test gave positive findings. Such an excess has also been reported in the presence of migraine (8), hypogonadism (9) and other conditions.

In this series, the Friedman test has been made in a selected group of 946 non-pregnant individuals and 209 positive reactions have been observed. Endocrine disease was suspected in each case in this group and this explains the large proportion of positive results. In 131 cases in the group, a clinical diagnosis of pituitary disease was made and excessive amounts of urinary prolan by means of this test were found in 59 of these cases. Among the cases diagnosed as pituitary disease, there were 15 cases of pituitary tumor and these form the basis of this paper.

In these 15 cases, the tumors have been verified at operation or autopsy in 8 instances and in the others the clinical findings were so definite that there was little doubt regarding the diagnosis.

ADVANTAGES AND DISADVANTAGES OF THE FRIEDMAN TEST

The Friedman test is an excellent qualitative method for prolan assay. The fact that the test is so widely used renders results, which are in any way unusual, of some importance. As in most other methods employed clinically, it does not distinguish clearly between the reactions produced by prolan A and prolan B, since the pituitary gland of the animal is intact; also, as in most other methods for prolan assay, the normal limits are not clearly defined. In spite of these disadvantages, it is generally accepted that a positive Friedman reaction usually indicates some abnormality. It is also true that when one individual has made over 1,100 such tests carefully, he may become expert in judging relatively between very marked, moderate, and slight reactions.

The finding of positive reactions to Friedman tests in non-pregnant conditions is important to all who are using the test as a method for the diagnosis of pregnancy. It is interesting also from the point of view of human pituitary physiology and in connection with the clinical management of tumors of the pituitary gland. It has been interesting to note that the clinical findings and histological characteristics of the tumors reported here are in no way constant, and that treatment of the tumor by such means as roentgenotherapy may be followed by considerable reduction in

the amount of urinary prolan. This raises the question of the probable value of prolan assays as a method for determining the effectiveness of treatment in such cases.

THE METHOD

For the test, adult, non-pregnant, female rabbits which weigh from 6 to 8 lb. are used. In our experience, rabbits that have had one or more litters of young are to be preferred to virgin females. The rabbit is isolated for 3 weeks and is then tested with urine known to contain an excess of urinary prolan. Either pregnancy urine or urine which has previously given strongly positive reactions is used for this purpose. If a positive response follows, the animal is a suitable test object after an interval of 10 days.

The urine for the test is a concentrated morning specimen, the patient's fluid intake having been restricted to 250 cc. after 4:00 P.M. on the day preceding the collection. The specimen is refrigerated, filtered to remove the precipitate which forms and, if alkaline in reaction, is acidified with HCl until the reaction is acid to litmus paper.

Four cc. of urine is injected intravenously into the test animal 3 times daily for 2 days. Under ether anesthesia and with aseptic technique, the abdomen is opened and the ovaries examined. The rabbit is considered ready for use again 10 days following a positive response.

A positive Friedman test reaction is indicated by the presence of either hemorrhagic or ruptured follicles, or by fresh corpora lutea. The urine from a normal individual may produce no response or a few mature follicles (not more than 2 in each ovary). In this work, the presence of 4 or more follicles in each ovary has been considered to indicate an excess of prolan, especially when the reaction in the ovaries is accompanied by swelling and congestion of the uterine horns, but is not spoken of as a positive reaction.

DIAGNOSIS OF PITUITARY TUMOR DISPROVEN

In addition to the 15 cases of pituitary tumor mentioned, 5 tests were made in 3 cases in which the preoperative diagnosis of pituitary neoplasm was disproven later. Positive test results were obtained in one of these cases and negative results in two. The positive finding was made in the case of a young man 24 years old, who had clinical evidence of hypogonadism. He shaved weekly, had very scanty pubic and axillary hair and an atrophic prostate gland. There was right hemianopsia and roentgen examination showed marked erosion of the posterior clinoid processes and depression of the sellar floor. The Friedman test showed 4+ corpora lutea. Autopsy revealed an oligodendroglioma of the floor of the third ventricle.

Of the two tests with negative results which were obtained in cases of disproven pituitary tumor, one occurred in a patient with pituitary dwarfism, hypogonadism and marked erosion of the sella turcica as shown by roentgen examination. Operation proved this to be due to pressure from a dilated third ventricle. In the second disproven case in which a negative test result was obtained, operation revealed a papilloma of a Rathke pouch cyst instead of pituitary tumor as suspected.

PITUITARY TUMOR WITH NEGATIVE FRIEDMAN TESTS

The 7 cases of pituitary tumor in which negative Friedman reactions were obtained will be described very briefly. The diagnosis of tumor was verified in 4 of these cases—at autopsy in one and by operation in 3. In the first two cases there was a questionable excess of prolan but, since the reactions were not positive, these are included in this group. In the remaining cases the Friedman tests gave completely negative results.

Case 1. A woman, 44 years of age, had extreme acromegaly, a large adenomatous goiter, hyperthyroidism with B.M.R. of +50 per cent, arterial hypertension, diabetes mellitus and in addition, amyotrophic lateral sclerosis. Estrin assays on the urine showed 20 R.U. in a 24-hour specimen (Kurzrok's method). Autopsy revealed a large acidophilic adenoma of the pituitary. The Friedman test was made preoperatively and gave a negative result although an excess of prolan was suggested by the 2+ increase in mature follicles produced in the ovaries of the test animals. Radiation therapy had been administered 2 months prior to the test.

Case 2. A young woman, 26 years of age, complained of amenorrhea which had been present for 5 years and of somnolence and fatigue. Obesity was especially marked about the trunk, over the upper dorsal spine and under the chin. No hirsutism, striae, hyperglycemia or polycythemia was present. The blood pressure was 100/74 mm. The sella turcica was eroded and there was blindness in the right eye and left hemianopsia. Operation revealed an adenoma almost entirely basophilic. The Friedman test made preoperatively gave a negative result, although a 2+ increase in ripe follicles was seen.

Case 3. A man, 54 years of age, had blindness in the right eye and left temporal hemianopsia. He was markedly obese. Hypogonadism was indicated by the marked hairlessness of the trunk, face, and limbs and the atrophic prostate, although the testes were normal in size and consistency. Adenoma of the pituitary was found at operation in 1926 and a small piece of the tumor was removed. Radiation therapy was given subsequently. The Friedman test was not made until 1935.

Case 4. A woman, 44 years of age, complained of weight loss and fatigue. The menses were normal. Bitemporal hemianopsia was present. Operation revealed a cystic adenoma of the pituitary with large numbers of basophil and acidophil cells. The prolan assay was made prior to operation.

Case 5. A woman, 35 years of age, complained of amenorrhea which had been present for 1 year. The body hair was scant, libido was diminished and the uterus was small. There was left temporal hemianopsia and erosion of the sella turcica as shown by roentgen examination. The B.M.R. was —25 per cent, the glucose tolerance was normal and an estrin assay (Kurzrok's method) yielded less than 4 R.U. in a 24-hour specimen of urine. The presence of a tumor was not verified by operation.

Case 6. A man, aged 34 years, complained of generalized convulsions. Ophthalmologic examination showed no abnormalities but the sella turcica was large and deep and the posterior clinoid processes were eroded. The glucose tolerance showed the curve of potential diabetes mellitus. An encephalogram revealed no abnormalities. The diagnosis of tumor was not verified by operation.

Case 7. A woman, 38 years of age, complained of headaches. Arterial hypertension was evidenced by a blood pressure of 170/110 mm. An operation for adenoma of the thyroid with hyperthyroidism had been performed when the patient was 22 years old. Examination revealed marked acromegaly and mild diabetes mellitus. Roentgen examination of the sella turcica showed pronounced erosion of the sellar walls. The diagnosis of adenoma of the pituitary gland was not verified.

PITUITARY TUMOR AND POSITIVE FRIEDMAN REACTIONS

Of the 8 cases of pituitary neoplasm in which positive Friedman test results were found, the diagnosis was verified in 4—by autopsy in 3 (cases 9, 11, and 12) and at operation in one (case 13). In cases 8 and 10, the clinical evidence of tumor was very clear. In cases 14 and 15, the evidence indicating the presence of pituitary tumor was less striking. In these cases there was,

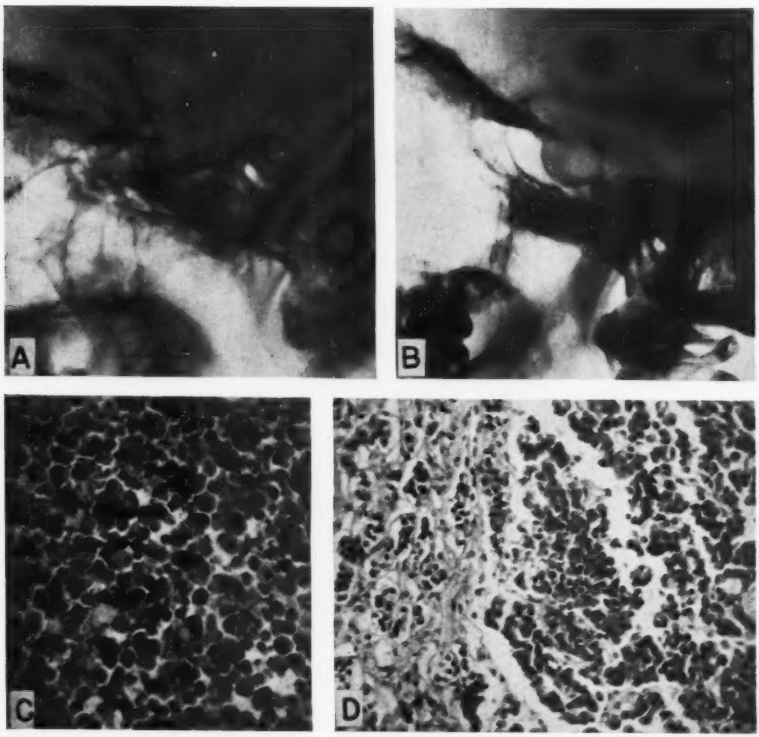


Fig. 1. *A*, Case 8: note marked erosion of posterior clinoid processes and floor of the sella. *B*, Case 10: note erosion of the dorsum of the sella, which is enlarged, round and deep. *C*, Case 9: section of pituitary gland. *D*, Case 11: section of pituitary; the tumor is on the right.

however, outspoken clinical acromegaly; enlargement of the sella turcica was present in both cases although there was no distinct bony erosion of the sellar boundaries and no visual field changes.

The first 4 cases in this group represent 3 distinctly different types of pituitary disease and are described in some detail. In the other 4 cases, only the essential findings are reported.

Pituitary Tumor with Acromegaly

Case 8. A young man, 29 years of age, was examined in November 1933. The following symptoms and signs of pituitary tumor were present: *a*) bitemporal, frontal, and occipital headaches of increasing severity which had been present for 4

months; *b*) bitemporal hemianopsia for form and color; *c*) paralysis of the left extra-ocular muscles with exception of the lateral rectus and ptosis of the left upper lid; *d*) haziness of the optic discs; *e*) marked erosion of the posterior clinoid processes and depression of the floor of the sella turcica as revealed by roentgen examination (fig. 1, *A*).

It was noted clinically and radiologically that there was enlargement of the frontal sinuses. Energy and endurance had diminished, and libido and potency had decreased steadily for 3 years. There was no obesity or obvious abnormality of the skin, hair or nails. No clinical evidence of thyroid disease was found, and the B.M.R. averaged —3 per cent. The spinal fluid was within the normal limits of pressure and gave normal findings on microscopical, chemical, and serological examinations.

The pituitary tumor, which obviously was associated with early acromegaly, was treated by roentgenotherapy. The headaches diminished and disappeared, the ocular

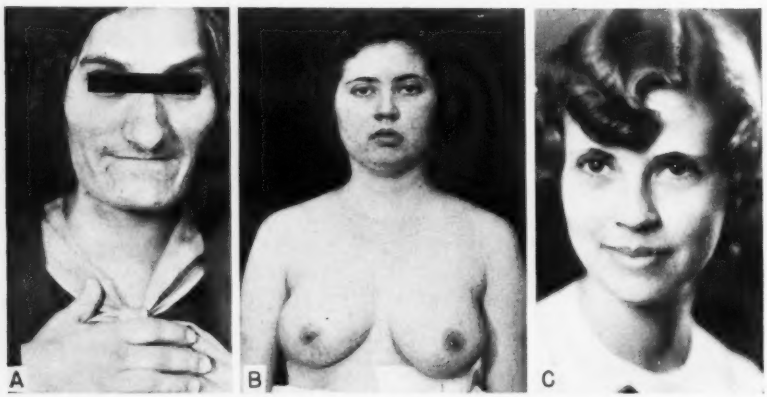


Fig 2. *A*, Case 9: note marked acromegalic features. *B*, Case 11: patient aged 17 years, prior to adrenal surgery. *C*, Case 11: same patient as in *B*, 18 months after left adrenal denervation and 14 months after right hemiadrenalectomy.

palsies were markedly improved, the visual fields gradually approached normal and libido and potency returned. During the course of treatment, hypometabolism and clinical signs of hypothyroidism responded to thyroid therapy and secondary anemia was controlled by iron therapy.

Before the first roentgenotherapy was administered, Friedman tests gave strongly positive findings—hemorrhagic follicles and many corpora lutea were produced. Following treatment, the amount of urinary prolan diminished and, according to this test, was absent for a period of six months. During this interval, a second smaller dose of x-ray had been given. Six months after the first treatment, the Friedman test again gave a positive result. Although fully positive findings were not present immediately prior to the third course of roentgenotherapy, a 2+ increase of mature follicles was present on 2 occasions. Following further therapy, the prolan again disappeared from the urine.

Before the administration of roentgenotherapy, testicular hormone was found to be present in the urine by our capon comb-growth method (9) in amounts distinctly above the average levels which were being found in normal persons by the method of extraction in use at that time. This also gradually decreased and finally fell to levels below the average normal.

It is interesting to note these changes in the presence of clinical findings which ordinarily would be interpreted as due to an acidophilic adenoma. The cellular structure of this tumor was not verified. The dates and amounts of radiation therapy, and the dates and results of the assays are given in tables 1 and 2.

TABLE 1. RESULTS OF PROLAN ASSAY BY FRIEDMAN TEST.

Case No.	Date and Amount of Roentgeno-therapy*	Date of Assay	Evidence of Amount of Prolan			
			Mature Follicles	Ruptured Follicles	Hemorrhagic Follicles	Corpora Lutea
8	11/17/33 to	11/17/33	−−	−−	+	++++
	11/24/33	12/ 4/33	++++	−−	−−	−−
	600 R	12/27/33	−−	−−	−−	−−
		1/27/34	−−	−−	−−	−−
	2/16/34 to	2/20/34	−−	−−	−−	−−
	2/22/34	5/19/34	++	+	+++	−−
	680 R					
		8/14/34	++	−−	−−	−−
	8/14/34 to	10/31/34	++	−−	−−	−−
	8/22/34	2/25/35	−−	−−	−−	−−
	630 R	5/25/35	−−	−−	−−	−−
9	6/18/34 to	5/23/34	+	++++	−−	−−
	7/ 3/34	6/30/34	+	−−	−−	+++
	800 R	8/ 2/34	−−	−−	−−	−−
10	4/29/33 to	4/24/33	++	+	+	−−
	5/ 2/33	6/26/33	++	−−	+	−−
	500 R	7/25/33	++	−−	−−	−−
		3/20/34	−−	−−	−−	−−
		10/ 2/34	−−	−−	−−	−−
11	Adrenal de-nervation,					
	5–31–33	5/31/33	+++	−−	++	−−
		6/ 4/33	+	−−	−−	+++
	10/ 6/33	6/ 9/34	−−	−−	−−	−−
		4/ 2/35	−−	−−	−−	−−

*Calculated as amount delivered to the hypophysis.

Case 9. A white woman (fig. 2, *A*), 35 years of age, complained of persistent, generalized, daily headaches which had been present for 12 months. Acromegalic facies had developed at adolescence. The menarche occurred at the age of 25 years and menses recurred only a few times, occasionally scanty, but at times profuse. During the few weeks preceding hospitalization, repeated attacks of menorrhagia were followed by weakness and pallor and from 10 to 15 lb. weight had been lost. There had been a craving for sweets for years and marked polydipsia and polyuria were present. A large adenoma of the thyroid had been present for 17 years.

The pulse rate was 108; the blood pressure was 155/90 mm. Basal metabolic rates were from 21 to 26 per cent above normal, but the blood iodine on 4 examinations was 8.1, 7.6, 11.3, and 9.0 micrograms per 100 cc., which is within normal limits. A glucose tolerance test, using 100 gm. of glucose orally, showed a fasting level of 147 mg. per 100 cc., and levels ½, 1, 2, 3 and 4 hours after glucose had been given of 280, 238, 214, 127 and 63 mg., respectively. The fluid intake was from 7 to 8 liters per day.

Roentgen examination revealed the skull to be markedly thickened and the sinuses enormous. There was a small area of calcification in the sella turcica. Pronounced

dorsal kyphosis was present. The optic discs were pale and the visual fields contracted concentrically. Rectal examination revealed no enlargement of the uterus, and no pelvic masses were present. Estrin assays showed 3 R.U. in a 24-hour output of urine. Curettement subsequently showed endometrial hyperplasia. Examinations of the blood showed 3,410,000 red cells and 36 per cent hemoglobin (Haden-Hausser). Transfusions of whole blood and iron therapy improved the condition of the blood.

TABLE 2. TESTICULAR HORMONE ASSAYS, CASE 8.

Date of Roentgenotherapy	Date of Assay	Average Comb-growth mm.
11/17/33 to 11/24/33	11/16/33	11.0
	12/27/33	16.0
	1/27/33	7.0
2/16/34 to 2/22/34	2/18/33	7.5
8/14/34 to 8/22/34	5/18/34	8.0
	8/17/34	6.0
	5/25/35	3.5

In the course of management, radiation therapy was applied to the pituitary gland in 9 doses between June 18 and July 3, 1934. Curettement of the uterus was followed by 578 mg.-hours of radium to the uterine canal. Subsequently, a severe manic psychosis developed and the patient died from bronchopneumonia 2 months later.

Friedman tests revealed the findings shown in table 1. At autopsy, the pituitary gland was found to be enlarged somewhat to the left, and on the right it overflowed the sellar boundaries and extended into the cavernous sinus. The sella was asymmetrically enlarged to the right. Microscopically the pituitary tumor was highly cellular with little stroma. The cells were large with a large amount of smooth acidophilic cytoplasm. There was a great pleomorphism of the nuclei. Many were large, vesicular, and rounded; many were deeply stained, irregular and eccentrically or peripherally placed, and double nuclei could be seen. Sections of the left portion of the pituitary body showed a small amount of tumor peripherally. In the anterior lobe, outside the tumor, large numbers of acidophilic cells were seen (fig. 1, *C*). (The pathological report on this pituitary gland was secured through the courtesy of Dr. H. S. Reichle, Cleveland City Hospital).

The next case illustrates another example of prolan excess in association with a tumor of the hypophysis. Attention is called to the fact that the clinical evidence suggests the presence of an adenoma of a nature entirely different from that in the first two cases, so far as its general hormonal effects are concerned. There has been no opportunity to study its cellular structure.

Pituitary Tumor with Hypogonadism

Case 10. The patient was a white girl, 19 years of age. Her chief complaints were of frontal and parietal headaches which had increased in intensity very slowly since childhood. Primary amenorrhea was present. Examination revealed the following signs of pituitary neoplasm: *a*) haziness of the optic discs; *b*) right temporal defect of the visual field approaching hemianopsia; *c*) enlarged, round, deep sella turcica with some erosion of the dorsum sella as revealed by roentgen examination (fig. 1, *B*).

Other important findings in the examination were that the weight was not

changing. The height was 64 in. and the weight 124 lb. Body fat was distributed normally. The external genitalia and secondary characteristics were not unusual. There was a tendency to male escutcheon of pubic hair and slight hypertrichosis of the upper lip, chin and nipples, but not distinctly more than is often seen in apparently normal individuals. The uterus was decidedly smaller than normal. There was no somnolence and no symptoms suggestive of disturbed water balance were found. The glucose tolerance was normal, and the B.M.R. was —15 per cent. The blood pressure and blood counts were normal. No urinary estrin could be measured by Kurzrok's method.

Roentgenotherapy to the pituitary gland was administered between April 29 and May 2, 1933. Following this, a gradual improvement in the visual fields occurred and 5 months later they became normal. The headaches diminished little if at all. Menstrual bleeding did not occur. The B.M.R. fell to —22 per cent and there was an increased tendency to gain in weight following this treatment.

Before radiation therapy, the Friedman test gave frankly positive findings. One cc. of Antuitrin-S had been administered 3 times a week prior to this time but none had been given for 2 weeks before the assay was made. In view of the rapid disappearance of immense quantities of prolan from the urine in cases of pregnancy a few days after delivery, it seems unlikely that the prolan injected therapeutically could explain the excess measured in this case. After such an excess was demonstrated, no further prolan therapy was used but the test still gave positive findings 55 days after the conclusion of radiation treatment. Prolan was again found in the urine in small amounts 84 days after radiation therapy but subsequently none was demonstrable by this test. The dates of assays and the dates and amounts of radiation therapy administered are shown in table 1.

Pituitary Tumor with Pituitary Adrenal Syndrome (Pituitary Basophilism)

Table 1 also summarizes the qualitative prolan assays in a typical case (Case 11) of pituitary basophilism (pituitary-adrenal syndrome) showing the disappearance of prolan from the urine following a left adrenal denervation and right hemi-adrenalectomy. Other cases of this type without proven tumor have been observed in which urinary prolan has disappeared following roentgenotherapy to the pituitary gland.

Case 11[1]. A girl, 17 years of age, was referred to us by Dr. Henry Turner of the University of Oklahoma and Dr. Henry Jacobs of Miami, Oklahoma. During the 2 years preceding our examination, her face had grown round and fat and there were accumulations of fat about the neck, over the upper dorsal regions and abdomen (fig. 2, *B*). A total of 20 lb. had been gained. Hypertrichosis had developed on the face, limbs, and back, and the pubic hair had assumed a male contour. She complained of heat intolerance, hyperhidrosis, palpitation, tachycardia and cold, sweating hands. Before her illness, she had led her classes and had been alert mentally, but since the onset of this disorder she had become dull and despondent and spoke of suicide. The menses had been normal from the age of 14 until 5 months before examination when oligomenorrhea developed.

The temperature was 99.4° F.; the pulse rate was 120, and the blood pressure was 130/95 mm. The B.M.R. was —1 per cent. Atrophic striae were present on the inner surfaces of the thighs and on the breasts which were abnormally large.

[1] This case was reported in greater detail in the New York State J. Med. 36: 475. 1936.

Examination of the blood showed 5,600,000 red cells with a hemoglobin content of 111 per cent.

On May 31, 1933, the Friedman test gave strongly positive findings—3+ mature follicles and 2+ hemorrhagic follicles. Denervation of the left adrenal gland was performed by Dr. George Crile on May 31, following which the symptoms improved and the B.M.R. fell to —14 per cent. Twenty-two days later, the Friedman test still gave positive findings, showing 1+ mature follicle and 4+ corpora lutea.

Right adrenal denervation and hemi-adrenalectomy were performed 4 months after the left denervation. Following a stormy convalescence during which signs of adrenal cortical insufficiency appeared, the patient made excellent progress. Her symptoms disappeared, the abnormal deposition of fat and hypertrichosis vanished and the menses became normal (fig. 2, *C*). Seven months later, no prolan could be demonstrated by the Friedman test, and 10 months later the test was frankly negative.

This patient died subsequently following an acute infection. Autopsy revealed an acute fibrinous pericarditis. The pituitary gland filled the sella turcica very tightly. The sella appeared normal except that its bony walls were very thin. The pituitary measured 1.4 x 1.4 x 0.7 cm. and weighed 1 gram. Grossly, it appeared slightly larger than average but was otherwise normal in appearance. Sections were made in a horizontal plane through the pituitary in such a manner that the stalk was included. On such a section, a well defined pars nervosa which was histologically normal was seen. This showed no basophilic infiltration. About two thirds of the entire anterior lobe of the pituitary was occupied by a well localized, oval-shaped nodule composed of a uniform type of cell which was almost entirely basophilic in staining reaction. Between this nodule and the pars nervosa, there was a rather wide zone of orderly arranged pituitary glandular tissue which appeared to be histologically normal (fig. 1, *D*). A section of this gland was sent to Dr. Percival Bailey who made a diagnosis of adenoma of the hypophysis, predominantly chromophobic in type with possibly basophilic and eosinophilic cells.

It is interesting to note in this case that there was a marked reduction in the amount of urinary prolan excreted following adrenal surgery alone when no treatment, which might explain this phenomenon, had been directed to the pituitary. The mechanism of such a change is obscure. The symptoms and signs were controlled by this method and later the presence of a distinct basophil (or chromophobe) adenoma of the pituitary was demonstrated. These findings appear to indicate that the adrenal gland is intimately involved in the production of the symptoms and signs of this disease.

Case 12. A woman, 28 years old, complained of drooping of the left upper eyelid and left external strabismus. She was obese and at the age of 20 years, amenorrhea had occurred for 3 years. Roentgen examination of the skull revealed erosion of the entire sella turcica. Estrin assays yielded 4.2 R.U. in a 24-hour specimen of urine. Autopsy showed a papillary carcinoma apparently arising in Rathke's pouch but since the tumor involved the pituitary gland, it is included in this group. The Friedman test, preoperatively, showed 2+ hemorrhagic follicles, 1+ mature follicles, 4+ swelling and 3+ congestion of the uterus of the test animals.

Case 13. A woman, 50 years of age, complained that her vision had become increasingly dim during the preceding year. There were mild headaches and somnolence and occasionally a mild uncinate attack. Examination of the visual fields

showed retention of vision only in the upper nasal quadrant on the left, and there was color loss on the right. The optic nerves were markedly atrophic. Roentgen examination of the sella turcica showed destruction of the posterior clinoid processes and some erosion of the anterior processes.

The patient's height was 65 in. and her weight was 154 lb. The blood counts, blood sugar, B.M.R. and blood pressure were normal. The Friedman test gave markedly positive findings, showing 3+ mature follicles and 4+ corpora lutea. At operation two separate tumors were removed by Dr. W. J. Gardner, one a meningioma from the right sphenoidal ridge and the other an adenoma of the pituitary. The latter was composed almost entirely of cells which stained intensively with hematoxylin. No acidophilic cells were seen.

Case 14. A woman, 34 years of age, had typical clinical acromegaly. Apart from a gain of 20 lb. in weight in 3 years, there were no neighborhood signs of pituitary tumor or hypothalamic damage except that roentgen examination revealed the sella turcica to be distinctly larger than average. The glucose tolerance and the B.M.R. were normal. The diagnosis of adenoma (probably acidophilic) has not been verified. Irradiation was directed to the pituitary gland in the following manner: Sept. 1933, 400 roentgen units; June 1934, 400 R.; June 1934, 400 R.; Sept. 1934, 800 R.; Aug. 1935, 1125 R.[2] Negative Friedman test reactions were found in Sept., 1933 and May, 1932; a frankly positive result in Aug., 1934 and again a completely negative result in Oct., 1934. Repeated estrin assays of the urine gave negative results.

Case 15. A man, 42 years of age, had well advanced acromegaly. There were headaches and an associated arterial hypertension as revealed by the blood pressure of about 200/120 mm. The B.M.R. and the glucose tolerance were normal. Visual fields showed no defects for form or color. Roentgen examination showed the sella turcica to be unusually large, but there was no evidence of bony erosion. The diagnosis of adenoma of the pituitary (probably acidophilic) has not been verified. In Sept. 1934, 975 R of x-ray was delivered to the pituitary gland. The Friedman reaction was negative prior to and during this treatment although a 2+ increase in follicles was seen. In Jan. 1936, the acromegaly had advanced noticeably and the Friedman reaction was positive, showing 1+ mature follicles and 4+ corpora lutea. In Sept. 1934, assay for testicular hormone showed 16 and 14 mm. comb growth in 2 caponized roosters and in Dec. 1934, only 6 and 8 mm. growth was obtained.

It is interesting in connection with the cases cited above to note that the type of tumor appears to bear no distant relationship to the prolan excess. This raises the important question of the cellular origin of gonadotropic substance. We have no evidence to indicate that prolan arises from any particular cell. The evidence does indicate that the prolan measured arises from the pituitary tumor however, since roentgen irradiation of such tumors was followed by a decided reduction in prolan excretion. It seems most unlikely that the associated hypogonadism which was present in some of the cases could be causing the excess of prolan since the hypogonadism has itself almost certainly been the result of pituitary dysfunction. It is clearly recognized that considerable variation in hormone production occurs, according to the methods employed in these cases, where no irradiation has been applied to the pituitary. To determine the consistency of such observations the study of much larger groups of cases and the use of more quantitative methods will be required.

[2] These doses are calculated as the amounts delivered to the gland.

SUMMARY

Positive Friedman test results have been observed in 8 of 15 cases of pituitary tumor. The 7 cases in which negative Friedman test results were obtained are described briefly. The diagnosis of tumor was verified at operation or autopsy in 4 instances and histological descriptions are available in 3; one was an acidophilic adenoma, one a basophilic adenoma and the other a cystic adenoma which contained large numbers of both acidophilic and basophilic cells.

Of the 8 cases in which positive reactions were obtained, the presence of tumor was verified at operation or autopsy in 4 instances. Two were basophilic adenomas, one of which was found in a patient with the syndrome of pituitary basophilism; one was an acidophilic adenoma, and one a papillary carcinoma of Rathke's pouch involving the pituitary. Of the unverified tumors, 3 occurred in patients with acromegaly and therefore these tumors were probably acidophilic adenomas; a fourth was associated with signs of hypogonadism.

In cases of pituitary tumor in which the Friedman test reaction is positive, radiation therapy to the pituitary may be followed by marked diminution in the excretion of prolan as judged by this test; in two instances, this has been accompanied by a diminution in the excretion of testicular hormone.

REFERENCES

1. Aschheim, S.: J.A.M.A. **104:** 1324. 1935.
2. Fluhmann, D. R.: J.A.M.A. **92:** 1744. 1929.
3. Mazer, C., and J. Hoffman: J.A.M.A. **96:** 19. 1931.
4. Fels, E.: Zentralbl. f. Gynäk. **53:** 466. 1929.
5. Aschheim, S.: Am. J. Obst. & Gynec. **19:** 335. 1930.
6. Ferguson, R. S.: J.A.M.A. **101:** 1935. 1933.
7. Hirsch-Hoffmann, H. U.: Klin. Wchnschr. **11:** 94. 1932.
8. Riley, H. A., R. M. Breckner, and R. Kurzrok: Bull. Neurol. Inst. New York **3:** 53. 1933.
9. McCullagh, E. P., D. R. McCullagh, and N. F. Hicken: Endocrinology **17:** 49. 1933.

THE SITE OF FORMATION OF THE POSTERIOR LOBE HORMONES

CHARLES FISHER
Institute of Neurology, Northwestern University, Medical School
CHICAGO, ILLINOIS

One of the long unsolved problems of endocrinology which has persistently defied the attacks of many investigators has been that of the site of formation of the posterior lobe hormones. It has generally been accepted that either the pars nervosa or the pars intermedia of the hypophysis represents the seat of elaboration of the pressor, antidiuretic and oxytocic principles, most workers favoring the pars intermedia because of their reluctance to concede that an organ of purely nervous origin, such as the pars nervosa, could have an endocrine function. But the pars tuberalis has not lacked champions, the most recent of whom has been Biggart (1), and the problem has been further complicated by the assigning of a secretory function on the part of some investigators to the cells of the tuber cinereum (2). The difficulty has been, of course, that in the ordinary laboratory animals the pars nervosa is encapsulated by the pars intermedia and the relationship is such a close one that it has not been possible to effect a separation between them for experimental purposes. Moreover, the pars tuberalis which forms a collar around the infundibulum has served as another obstacle in the interpretation of experiments involving this region.

Recently Geiling and his co-workers have presented what is probably the most convincing evidence thus far published that the pars nervosa, entirely independent of the pars intermedia, elaborates the physiologically active components of the posterior pituitary. De Lawder, Tarr and Geiling (3) reported that in the chicken hypophysis, which has no pars intermedia, the pars nervosa contains pressor, oxytocic and anti-diuretic components, and no melanophore principle, while the latter is extractable from the anterior lobe. Later, Geiling (4) obtained similar results from the hypophyses of the finback and sperm whales, forms which likewise do not possess a pars intermedia. Further, Geiling and Lewis (5) prepared tissue cultures of the separate lobes of mouse and rat pituitaries. They found that material derived from the cultures of the pars intermedia possessed no blood-pressure raising property, but had a marked melanophore-expanding effect; the cultures of the anterior lobe had no pressor or melanophore effects; while the cultures of the neural lobe admixed with a ragged edge of pars intermedia had both pressor and melanophore effects. They concluded that the evidence derived from these tissue culture experiments supports the view that the pars intermedia elaborates the melanophore-expanding hormone, but further indicates that the pressor hormone is not formed by this division of the pituitary.

During the course of an investigation on the relation of the hypophysis

and the hypothalamus to water metabolism in the cat, we have accumulated evidence which indicates that the integrity of the pars nervosa is necessary for the elaboration of the so-called posterior lobe hormones. The results to be reported here are of particular interest since they were obtained from one of the ordinary laboratory mammals, the cat, which has a well developed pars intermedia.

It has been shown that diabetes insipidus can be produced in the cat and monkey by placing lesions in the anterior hypothalamus so as to interrupt the supraoptico-hypophyseal tracts (6, 7, 8, 9) or by completely extirpating the posterior lobe or cutting the stalk (10). When such lesions are made in the hypothalamus the pars nervosa of the hypophysis and the supraoptic nuclei of the hypothalamus undergo marked degenerative changes. The entire posterior lobe shows a considerable decrease in size and this is accompanied by a widening of the interglandular cleft and a marked increase in size of the posterior lobe cavity. The walls of the stalk become much thinner and the space between them much wider. Microscopically, it has been found that the unmyelinated fibers of the supraoptico-hypophyseal tracts have undergone degeneration and can no longer be demonstrated, with the exception of a few fibers, in the walls of the stalk and in the pars nervosa. At the same time, there appears to be a degeneration of the glial-like cells (pituicytes) of the pars nervosa. As compared to the normal, the atrophic pars nervosa shows a hypercellularity. These cells are not normal pituicytes but perhaps connective tissue cells or some other element, the exact nature of which has not been determined. Figure 1, *A*, shows the pituicytes and the network of glial fibers in the pars nervosa of a normal cat, figure 1, *B*, the absence of this network and the dense accumulation of cells without processes in the atrophic pars nervosa of a cat with diabetes insipidus.

We have been impressed by the fact that these degenerative changes appear to be confined to the pars nervosa, the pars intermedia being histologically intact, showing only a wrinkling and folding to accommodate itself to the shrinkage of the nervous portion of the gland. Now, it has been shown by means of appropriate assays that these atrophic glands are almost entirely lacking in antidiuretic, pressor and oxytocic activity, the loss of the antidiuretic activity probably being the cause of the diabetes insipidus (11). In preparing the extracts for these assays both anterior and posterior lobes were utilized but, in spite of the presence of normal pars intermedia tissue, none of the active components just mentioned were present in any significant amounts. It would seem that what we have here is an atrophic, non-functional pars nervosa surrounded and encapsulated by a histologically normal pars intermedia. In order to put this assumption to the test, it was decided to assay these atrophic glands for their melanophore-expanding activity, which would presumably demonstrate whether or not the pars intermedia was functioning. If it could be shown that the pars intermedia was physiologically active as well as histologically intact it would mean that the absence of the other components of similarly atrophic posterior lobes, namely, the pressor, antidiuretic and oxytocic principles, was associated with the degeneration of the pars nervosa.

The rationale of this experiment depends upon the correctness of the view that the pars intermedia elaborates the melanophore-expanding principle. It may be said that there is practically universal agreement, based on an impressive mass of experimental evidence, that this is the case. Atwell (12) temporarily restored silvery hypophysectomized frog tadpoles to approximately the normal dark condition by submitting them to the action of posterior pituitary or of pure pars intermedia solutions. Smith (13) was able to confirm these observations and Allen (14) and Swingle (15) reported the restoration of the dark condition in hypophysectomized tadpoles by means of pars intermedia implants. Hogben (16, 17) has presented further evidence that the pigmentary effector system in amphibia is under the control of the pars intermedia. Allen (18) definitely concluded that the pigmentary hormone of the amphibian hypophysis is produced only by the pars intermedia. Van Dyke (19) showed that the pars intermedia of cattle contains a much greater amount of melanophore-expanding substance than does any other part of the hypophysis. Mention may be made of the interesting report of Bayer (20) who studied a white frog found in a shipment of 70 specimens of Rana esculenta. Serial sections revealed that the pars intermedia had been replaced by an encysted parasite. More recently, Atwell and Holley (21) removed from $\frac{1}{3}$ to $\frac{2}{3}$ of the caudal portion of the epithelial hypophysis of Rana sylvatica tadpoles from which the pars intermedia develops at the tail-bud stage. These tadpoles became silvery but underwent metamorphosis and it is concluded that they became silvery because they lacked the pars intermedia but metamorphosed because there was sufficient anterior lobe tissue left to induce adequate stimulation of the thyroid gland. The absence of the pars intermedia as a result of removal of its anlage was demonstrated in serial sections. Anderson and Haymaker (22) in some interesting tissue culture experiments were able to demonstrate that with growth in vitro pars intermedia cells retain their power to elaborate the melanophore-expanding principle. Mention has already been made of the experiments of Geiling and Lewis (5) in which it was shown that isolated pars intermedia tissue growing in vitro produced the pigmentary hormone but no pressor principle. In those forms which lack a pars intermedia or have only a rudimentary one the melanophore principle appears to be formed in the anterior lobe. Thus, De Lawder, Tarr and Geiling (3) found this to be so for the chicken and Geiling (4) obtained the same result with the whale. Roth (23) and Jores and Glogner (24) report that in man the melanophore hormone is elaborated by the anterior lobe and probably by the basophile cells.

In addition to the melanophore tests an attempt was made to assay these atrophic glands for their intermedin content or erythrophore-expanding activity. Since Zondek and Krohn (25) report that this principle is formed in the pars intermedia we would thus have another test for pars intermedia function.

METHOD

Extracts were prepared from the pituitaries of 5 normal adult cats and from the atrophic glands of 4 adult cats which had diabetes insipidus. The diabetes insipidus was produced in these animals (cats D54, D59, D64 and D66) by placing lesions in the anterior hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument so as to interrupt the supraopticohypophyseal tracts in the same manner as described elsewhere (6). The operations were performed by Dr. Ingram. The onset and development of the disturbance in water metabolism in these animals were the same as those

described in previous papers (6, 7, 9). Table 1 gives an indication of the intensity of the disturbance of water balance in these cats; it should be borne in mind that the fluid exchange of normal cats amounts to about 100 cc. daily and the specific gravity of the urine to around 1.030. After the polyuria and polydipsia had been present for several weeks and while they still persisted the animals were killed. All the cats, the operated and the normal, were

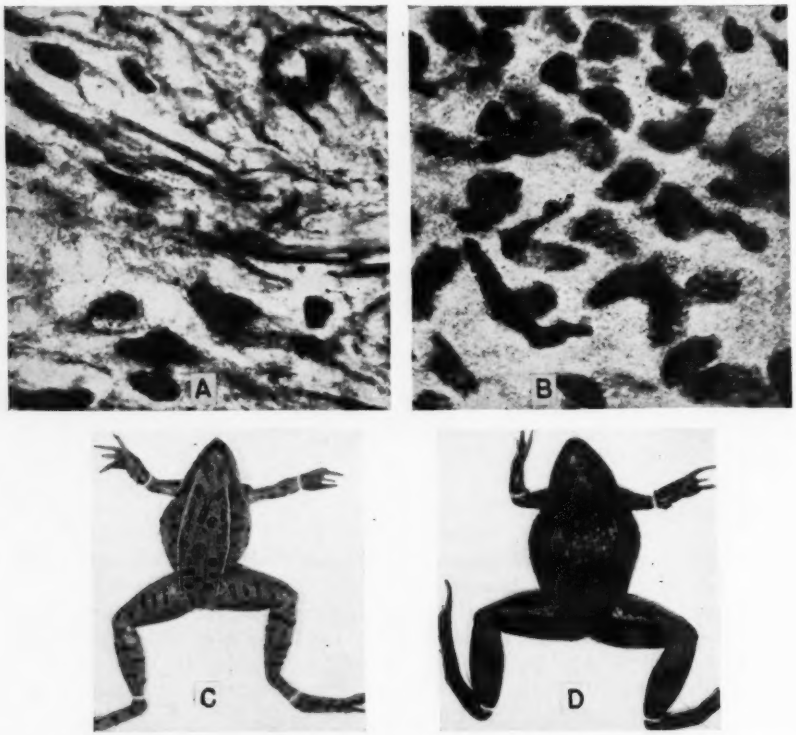


Fig. 1. *A*, Photomicrograph of the pars nervosa of a normal cat. Note the long processes on the pituicytes and the glial fibers in the background. Penfield modification of the del Rio-Hortega silver carbonate stain. *B*, Photomicrograph of the pars nervosa of a cat with diabetes insipidus. Note the absence of processes on the cells, the absence of glial fibers in the background and the hypercellularity. Same stain.

C, Photograph of frog XI, injected with 0.2 cc. of a neutral control solution and showing no expansion of the melanophores. *D*, Photograph of frog X2, injected with 0.2 cc. of the extract, prepared from the atrophic gland of cat D64, one hour and forty minutes before. The melanophore expansion is maximal.

killed by bleeding and the glands removed within ½ hour after death. The hypophyses were cut away from the tuber cinereum with a sharp scalpel, the incision being made deep enough so that all parts of the pituitary, including the stalk and pars tuberalis, were obtained. The glands were immediately placed in acetone, desiccated and later pulverized.

The acetone-dried powders of the whole glands of cats D54 and D59 were extracted with 10 cc. of 0.25 per cent acetic acid, those of cats D64 and D66 with 10 cc. of N/10 NaOH, by boiling for 5 minutes, according to the methods described by Jores and Glogner (24) and Jores and Lenssen (26).

These investigators report that acetic acid extraction gives a greater yield in erythrophore-expanding hormone, while NaOH gives a greater yield in melanophore-expanding substance. After the extraction the solutions were neutralized, using brom-thymol-blue as an indicator and the final volume was made up to 10 cc. in the cases of D64 and D66 and to 5 cc. and 40 cc., respectively, in the cases of D59 and D54. Two separate extracts were prepared from the gland of D64, one made from the greater part of the anterior lobe and the other from the remaining portion of the anterior lobe and the stalk, pars tuberalis and pars nervosa. Similarly, for the normal glands several acid and several alkaline extracts were prepared.

In order to determine the presence or absence of activity of the pars intermedia all 4 extracts of the glands of the polyuric cats were tested for their melanophore-expanding power on the live frog. In addition, the greater part of the extract of the pituitary of cat D59 was tested for its erythrophore-expanding activity. In view of the difficulty of obtaining the fish, Phoxinus laevis, which Zondek and Krohn (25) specify as the test-object for intermedin, we used the 'red bellied dace.' This fish, Chrosomus erythrogaster, belongs to the group of Cyprinidae which also includes Phoxinus.[1] The so-called 'wedding dress' of this fish is visible during the breeding season and consists of a vivid and brilliant red discoloration of the otherwise white belly of the male and the part just below the mandible on each side. Saphir (27, 28) was the first to artificially produce the 'wedding dress' in the 'red-bellied dace' by means of posterior pituitary extracts and his results have been confirmed by Young (29).

The frogs used for the melanophore test were kept in large glass jars on a white background, the bottom of the jars containing a small amount of water or covered with cotton which was kept moist. Before any series of injections, an attempt was made to have the frogs approximately the same shade of coloration. The experiments were carried out in late summer and early fall and the frogs were kept in a position to get as much sunlight as possible.

Injections in varying amounts and dilutions were made into the dorsal lymph sacs of the frogs, using a tuberculin syringe. The frogs were then observed for any change in coloration over a period of hours. We were interested not only in a qualitative test of the melanophore-expanding activity of these extracts but also in obtaining a rough quantitative comparison between the normal and atrophic gland extracts. This was done by gradually increasing the dilutions and comparing the effects obtained.

The manner of caring for and the method of injection of the 'red-bellied dace' utilized have been described by Saphir (27). The effects of the injection of varying amounts of the extract of the gland of D59 were compared with those elicited with the extracts of normal glands. A control solution composed of the reagents used in extracting the glands was prepared (an acetic acid-sodium hydroxide solution brought to the neutral point with brom-thymol-blue). The effect of injecting this was tested on both frogs and fish.

[1] We wish to express our thanks to Dr. William Saphir for his advice and suggestions on the use of the 'red-bellied dace'.

RESULTS

Melanophore-Expanding Test. Altogether 30 injections were made with the 4 extracts of the atrophic glands of the polyuric cats: 4 with D54, 6 with D59, 8 with D66 and 12 with D64. It may be said at once that melanophore expansion was obtained with all the extracts with doses ranging from 1 cc. of the original solutions to 0.1 cc. of dilutions as high as 1:10,000. The degree of expansion was roughly proportional to the dose and the dilution. In agreement with Jores and his co-workers, it was found that the alkaline extracts gave much better effects than did the acid. Moreover, so far as we could observe, the extracts from the atrophic glands gave qualitatively and quantitatively as good results as did the extracts of the normal glands.

The results obtained with the 4 extracts were essentially the same so that a more detailed description will be given only for the extract of the gland of D64. The results in this case are of particular significance because D64 had the most marked polyuria and polydipsia of the 4 cats and, therefore, presumably the greatest degree of degeneration of the pars nervosa. Nevertheless, the extract prepared from this atrophic gland displayed as much melanophore-expanding activity as did any of the other extracts used. The following protocol will give an indication of the results obtained.

October 21, 1935:

10:00 A.M. Frog X2 injected with 0.2 cc. of undiluted D64 extract. Before injection this frog had same color as the control, X1, shown in figure 1, *C*.
10:10 A.M. Has become quite dark
10:30 A.M. Has become coal black
11:45 A.M. Still very black (photograph taken)
12:30 P.M. Still very black
5:40 P.M. No appreciable fading yet

October 22, 1935:

10:00 A.M. Still considerably darker than the control

As a control, frog X1 was injected with 0.2 cc. of the neutral control solution. At no time did it show any change in color. Figure 1, *C, D,* are photographs of frogs X1 and X2 showing the high degree of melanophore expansion in the latter 1 hour and 45 minutes after the injection and the absence of any effect in X1.

As can be seen, very marked reactions, lasting for 24 hours or more, could be obtained with several tenths of a cubic centimeter of the undiluted solutions of the atrophic glands. These were maximal reactions and so far as we could tell they came on as quickly and persisted as long as those elicited with extracts prepared from the normal glands. Dilutions as high as 1:10,000 were made of the extract of D64 and the reactions obtained compared with those observed with similar dilutions of extracts of normal glands. In one experiment, for example, frogs injected with 0.1 or 0.2 cc. of a 1:10,000 dilution became quite dark, though not as dark as X2 in figure 1, and the reaction was still present after 3 hours. Again the extract of the atrophic gland gave as good results as that of the normal gland. With the extract prepared from the greater part of the anterior lobe of D64 a slight to moderate reaction could be obtained of the same order of magnitude as those obtained with similar extracts prepared from normal glands.

Erythrophore-Expanding Test. The results noted after injecting the 'red-bellied dace' will be discussed very briefly. It will suffice to point out that with doses of 0.1 to 0.2 cc. of the undiluted acid extract of the atrophic gland of D59 a brilliant display of the 'wedding dress' could be elicited, the reaction coming on within 10 to 30 minutes and persisting from several hours to 24 hours. The reaction was as prompt and as marked and persisted as long as those obtained when extracts of normal glands were injected. However, in our hands, this test gave very capricious results with control solutions. In a number of instances good reactions were obtained after the injection of

TABLE 1. FLUID EXCHANGE DURING TYPICAL 2 WEEKS OF CATS WITH DIABETES INSIPIDUS.

Cat No.	Average Daily Urine Output cc.	Average Daily Specific Gravity of Urine	Average Daily Fluid Intake[1] cc.
D64	616	1.008	688
D54	381	1.010	371
D59	245	1.014	332
D66	171	1.016	241

[1] This includes 100 cc. of milk per day.

neutral solutions, distilled water or even air. Moreover, we have observed spontaneous reactions which persisted for some time. Because of this the test was abandoned and the other extracts were used exclusively on the frogs.

DISCUSSION

The demonstration of a normal melanophore hormone-content in the atrophic neuro-intermediate lobes of cats with diabetes insipidus indicates that the pars intermedia of these glands has not lost its activity. Since the pars intermedia appears to be physiologically active as well as histologically intact, one is justified in drawing the conclusion that the absence of antidiuretic, pressor and oxytocic activities is correlated in some way with the degeneration of the pars nervosa. The results would seem to indicate that the pars nervosa itself takes part in the elaboration of these components, although it is not known just what elements in this organ are capable of secretory activity. One possibility is that the pituicytes may play a rôle in the elaboration of the posterior lobe hormones and it is of interest to note that in the atrophic pars nervosa these glial cells appear to have degenerated (compare fig. 1, *A* and *B*). The fibers of the supraoptico-hypophyseal tracts end around the pituicytes and would seem to exert a trophic influence over them, for, when these fibers degenerate, the pituicytes likewise degenerate. That the pars nervosa might have an endocrine function should not be so astonishing in view of the generally accepted fact that adrenalin is produced by the suprarenal medulla, an organ which also is of neural origin.

Recent work has thrown doubt on the theory that the hyaline bodies of Herring represent precursors of the active components of the posterior pituitary. Gersh and Tarr (30) have adduced convincing evidence in a number of mammals that the hyaline bodies of Herring are artefacts which arise

from the homogeneously distributed protein that infiltrates uniformly the glial network of the fresh pars nervosa. Further, in the light of the investigations of Wislocki and Geiling (31) it seems improbable that these hyaline bodies originate from the pars intermedia. These workers were able to show that hyaline bodies, histologically similar to those found in other mammals, are present in the neural lobes of 4 different species of whale. Since, in the whales, anterior lobe and neural lobe are anatomically separate and, moreover, there is no pars intermedia, they concluded that these bodies originate in the whales in the neural lobe.

Another theory concerning the site of formation of the posterior lobe hormones may be mentioned here. Gaupp and Scharrer (2), on purely histological grounds, have come to the conclusion that the supraoptic nuclei not only regulate the activity of the posterior lobe but are themselves capable of secreting hormones. They suggest that in the absence of the hypophysis these nuclei take over the antidiuretic function of the posterior lobe. It should be pointed out that this is hardly possible in view of the fact that these nuclei uniformly undergo marked degeneration whenever the pars nervosa is extirpated.

The results reported here do not obviate the often expressed possibility that the pars intermedia may elaborate precursors which are in some way taken up by the pars nervosa and transformed into the active components. They demonstrate only that the pars nervosa is necessary for the complete elaboration of the latter. However, the results of Geiling and his co-workers make it seem highly probable that the pars intermedia takes no part in the formation of the known posterior lobe principles except that acting on pigmentation.

The accumulated evidence of previous workers has always suggested that the pars nervosa was the site of formation of the pressor, antidiuretic and oxytocic components. Thus, Howell (32) found that the pressor principle was present only in the pars neuralis; Osborne and Vincent (33) reported that the pars intermedia contains less pressor hormone than the pars nervosa; Herring (34), Hogben and de Beer (35) and Van Dyke (19), likewise, found that the pars nervosa contained greater amounts of the active components than did the pars intermedia.

Our results on the erythrophore-expanding activity of the extract of the gland of cat D59 are presented for what they are worth. If they can be accepted they furnish further evidence for the functional integrity of the pars intermedia. It may be noted that Stutinsky (36) and Collin and Drouet (37) have reported positive results with this test after the injection of thyroid, orchitic, placental, ovarian, thymic and myocardial extracts, using Phoxinus laevis as the test-object. It does not appear to be a very reliable method and Jores and Lenssen (26) are of the opinion that the melanophore test is much superior to it.

At any rate, there is no doubt that the extracts of these atrophic glands contain a substance which is capable of expanding the erythrophores of the dace. Although not specific, this substance gave much better and longer-lasting reactions than did the control solutions and with uniform regularity. These

results would suggest that the substance which expands the pigment cells of the dace and the antidiuretic principle are not identical, for it has been shown (11) that atrophic glands of the same type as those used in the present investigation are lacking in antidiuretic activity. This is of some importance in view of the reports of Sulzberger (38), Ferguson (39), Turner (40) and Kahn (41) that they were able to suppress the polyuria and polydipsia of patients with diabetes insipidus with preparations of intermedin. On the other hand, Dischreit (42) was unable to confirm these observations on 3 polyuric patients. More recent work has been reported, however, which shows that intermedin and the antidiuretic hormone are not identical and that the antidiuretic effects obtained with the former were due to contamination by the latter. Thus, Sulzberger (43) retracted his former statements and reported that he has obtained intermedin preparations which had marked erythrophore-expanding activity on Phoxinus but no demonstrable antidiuretic effect. Similarly, at the suggestion of Ferguson, Downes and Richards (44) reinvestigated the problem and found that the antidiuretic effect of intermedin was due to contamination with pitressin and pitocin.

Indication that the antidiuretic component may have a different site of origin than the melanophore principle is furnished by the demonstration that these hormones are not identical. Evidence for this may be found in the papers by Dreyer and Clark (45), Rowe (46), Hogben and Gordon (47), Dietel (48) and Stehle (49).

The occurrence of degenerative changes in the pars nervosa after interruption of the supraoptico-hypophyseal tracts could not, of course, be checked microscopically in the glands which were used for assay purposes. We have, however, always found them to be present in the pituitaries of a large number of cats which developed diabetes insipidus. Moreover, the atrophy of the posterior lobe can be observed grossly, while the presence of marked atrophy of the supraoptic nuclei is a further indication that the pars nervosa has undergone degeneration. Recently Biggart (1) has reported degenerative changes in the pars nervosa and stalk, similar in nature to those found in our cats, and also degeneration of the pituicytes in the hypophysis of a patient with diabetes insipidus. It is interesting to note that Anderson and Haymaker (22) failed to observe any growth of the pars nervosa elements in their tissue culture experiments and at the same time there was no increase in the antidiuretic content of these in vitro growths. On the other hand, the pars intermedia tissue showed extensive proliferation with an 8-fold increase in the amount of melanophore hormone. The failure of the growth of pars nervosa elements and the absence of any increase in antidiuretic content in these glands which were necessarily separated from their hypothalamic innervation in the process of preparing tissue cultures is paralleled by the degeneration of the pars nervosa and the absence of antidiuretic hormone in our cats with diabetes insipidus. Likewise, Gardner and Hill (50) transplanted whole pituitaries into the testes of rats and found that while the anterior lobes persisted and appeared to be histologically intact, the posterior lobes uniformly underwent degeneration. In transplanting the hypophysis to the anterior chamber of the eye in

albino rats Buxton (51) also reported that while the anterior lobes persisted in a certain percentage of cases the posterior lobes degenerated.

A number of investigators (6, 52, 53, 54, 55) have pointed out that some of the fibers from the hypothalamico-hypophyseal tracts appear to penetrate into the pars intermedia. This would suggest that these fibers exert a regulating influence over the pars intermedia. However, in our experience the number of these fibers in the intermediate lobes of the cat, monkey and rat is very small and the fibers penetrate but a short distance. Although these fibers may have an influence on some function of the pars intermedia as yet unknown, they do not, at any rate, seem to be necessary for the secretion of the melanophore and erythrophore-expanding principles.

One further point needs to be discussed. The theory that the pars tuberalis secretes the antidiuretic hormone or that it may assume this function in the absence of the posterior lobe has received considerable recognition (1, 56, 57). In our experience, however, with 60 cats[2] with diabetes insipidus, the pars tuberalis may be perfectly intact without preventing the development of the disturbance of water metabolism. Also, in the assays of the atrophic glands of cats with polyuria which have been reported (11), the entire hypophysis was utilized, including the pars tuberalis, and yet no appreciable antidiuretic activity could be demonstrated. What little experimental work has been done on the pars tuberalis indicates that it has, not an antidiuretic function, but a diuretic one. Thus, Atwell (58) was able to elicit a pronounced diuresis with pars tuberalis extracts. Earlier, Atwell and Marinus (59) found that pars tuberalis extracts had very little pressor and oxytocic activity as compared to extracts prepared from the stalk and pars intermedia. Ingram and Barris (60) showed that direct stimulation of the anterior pituitary with the Horsley-Clarke stereotaxic instrument produced a diuresis. They found that such an effect occurred when the point of application of the stimulus was on the superficial aspect of the anterior lobe in close proximity to the pars tuberalis; while stimulation deep in the anterior lobe was not found to be associated with diuresis.

SUMMARY

Four atrophic posterior lobes of cats with diabetes insipidus were subjected to assay for their melanophore-expanding activity on the live frog. The results indicate that the pars intermedia of these glands is physiologically active as well as histologically intact. The conclusion is, therefore, drawn that the absence of pressor, antidiuretic and oxytocic activities in glands of the same type as those used in this study is correlated with the degeneration of the pars nervosa and that the latter must play a rôle in the elaboration of these components.

These results are discussed with reference to the various theories of posterior lobe hormone formation.

[2] These animals, including those utilized in this investigation, were operated upon by Dr. W. R. Ingram.

REFERENCES

1. Biggart, J. H.: Edinburgh M. J. **43:** 417. 1936.
2. Gaupp, R. Jr., and E. Scharrer: Ztschr. f. d. ges. Neurol. u. Psychiat. **153:** 327. 1935.
3. De Lawder, A. M., L. Tarr and E. M. K. Geiling: J. Pharmacol. & Exper. Therap. **51:** 142. 1934.
4. Geiling, E. M. K.: Bull. Johns Hopkins Hosp. **57:** 123. 1935.
5. Geiling, E. M. K., and M. R. Lewis: Am. J. Physiol. **113:** 534. 1935.
6. Fisher, C., W. R. Ingram and S. W. Ranson: Arch. Neurol. & Psychiat. **34:** 124. 1935.
7. Fisher, C., W. R. Ingram, W. K. Hare and S. W. Ranson: Anat. Rec. **63:** 29. 1935.
8. Fisher, C., and W. R. Ingram: Arch. Int. Med. **58:** 117. 1936.
9. Ingram, W. R., C. Fisher and S. W. Ranson: Arch. Int. Med. **57:** 1067. 1936.
10. Ingram, W. R., and C. Fisher: Anat. Rec. **66:** 271. 1936.
11. Fisher, C., and W. R. Ingram: Endocrinology **20:** 762. 1936.
12. Atwell, W. J.: Science **49:** 48. 1919.
13. Smith, P. E.: Am. Anat. Mem. No. 11, 1920.
14. Allen, B. M.: Science **52:** 274. 1920.
15. Swingle, W. W.: J. Exper. Zoöl. **34:** 119. 1921.
16. Hogben, L. T.: The Pigmentary Effector System, Oliver and Boyd, Edingburgh and London, 1924.
17. Hogben, L. T.: Proc. Roy. Soc., London, s.B. **120:** 142. 1936.
18. Allen, B. M.: Proc. Soc. Exper. Biol. & Med. **27:** 504. 1930.
19. Van Dyke, H. B.: Arch. f. exper. Path. u. Pharmakol. **114:** 262. 1926.
20. Bayer, G.: Endokrinologie **6:** 249. 1930.
21. Atwell, W. J., and E. Holley: J. Exper. Zoöl. **73:** 23. 1936.
22. Anderson, E., and W. Haymaker: Proc. Soc. Exper. Biol. & Med. **33:** 313. 1935.
23. Roth, A.: Centralbl. f. allg. Path. u. path. Anat. **54:** 234. 1932.
24. Jores, A., and O. Glogner: Ztschr. f. d. ges. exper. Med. **91:** 91. 1933.
25. Zondek, B., and H. Krohn: Klin. Wchnschr. **11:** 405; 849; 1293. 1932.
26. Jores, A., and E. W. Lenssen: Endokrinologie **12:** 90. 1933.
27. Saphir, W.: Proc. Soc. Exper. Biol. & Med. **31:** 864. 1934.
28. Saphir, W.: Arch. Path. **19:** 24. 1935.
29. Young, A. M.: Am. J. Clin. Path. **4:** 485. 1934.
30. Gersh, I., and A. D. Tarr: Anat. Rec. **63:** 231. 1935.
31. Wislocki, G. B., and E. M. K. Geiling: Anat. Rec. **66:** 17. 1936.
32. Howell, W. H.: J. Exper. Med. **3:** 245. 1898.
33. Osborne, W. A., and S. Vincent: Brit. M. J. **1:** 502. 1900.
34. Herring, P. T.: Quart. J. Exper. Physiol. **8:** 245. 1915.
35. Hogben, L. T., and G. R. de Beer: Quart. J. Exper. Physiol. **15:** 163. 1925.
36. Stutinsky, F.: Compt. rend. Soc. de biol. **115:** 241. 1934.
37. Collin, R., and P. L. Drouet: Compt. rend. Soc. de biol. **115:** 1441. 1934.
38. Sulzberger, M. B.: J.A.M.A. **100:** 1928. 1933.
39. Ferguson, R. S. Personal communication, 1934.
40. Turner, H. H.: Endocrinology **19:** 275. 1935.
41. Kahn, B. S.: Quoted by Sulzberger (43).
42. Dischreit, J.: Klin. Wchnschr. **14:** 629. 1935.
43. Sulzberger, M. B.: Klin. Wchnschr. **15:** 489. 1936.
44. Downes, H. R., and L. Richards: J. Biol. Chem. **110:** 81. 1935.
45. Dreyer, N. B., and A. J. Clark: J. Physiol. **58:** xviii. 1923-24.
46. Rowe, L. W.: Endocrinology **12:** 663. 1928.
47. Hogben, L. T., and C. Gordon: Quart. J. Exper. Biol. **7:** 286. 1930.
48. Dietel, F. G.: Klin. Wchnschr. **12:** 1358. 1933.
49. Stehle, R. L.: Arch. f. exper. Path. u. Pharmakol. **175:** 466. 1934.
50. Gardner, W. U., and R. T. Hill: Proc. Soc. Exper. Biol. & Med. **32:** 1382. 1935.
51. Buxton, C. L.: Anat. Rec. **64:** 277. 1936.
52. Croll, M. M.: J. Physiol. **66:** 316. 1928.
53. Cajal, S. Ramon y: Anales de la Sociedad Espanola Historia Natural, Serie II, Tomo Tercero, **23:** 195. 1894.
54. Tello, F.: Trabajos del Laboratorio de Investigaciones Biologicas de la Universidad de Madrid **10:** 145. 1912.
55. Bucy, P. C.: Penfield's Cytology and Cellular Pathology of the Nervous System, **2:** 705, Hoeber, New York, 1932.
56. Frank, E.: Med. Klin. **25:** 699. 1929.
57. Brull, L.: Presse Med. **41:** 1267. 1933.
58. Atwell, W. J.: Proc. Soc. Exper. Biol. & Med. **24:** 864. 1927.
59. Atwell, W. J., and C. J. Marinus: Am. J. Physiol. **47:** 76. 1918.
60. Ingram, W. R., and R. W. Barris: Endocrinology **19:** 432. 1935.

EXPERIMENTAL STUDIES OF THE ANTERIOR PITUITARY. IV. THE REPLACEMENT CAPACITY AND THE NON-CYCLIC BEHAVIOR OF HOMOPLASTIC ANTERIOR PITUITARY GRAFTS

M. SCHWEIZER, H. A. CHARIPPER AND H. O. HATERIUS[1]
Department of Biology, Washington Square College, New York University
NEW YORK CITY

In developing an attack upon certain aspects of anterior pituitary activity, the writers reported recently upon the viability and persistence of hypophyseal grafts (1). Briefly, it was shown that intra-ocular transplants (homoplastic) may display a remarkable survival capacity, and that tissue so grafted may retain its histological differentiation for periods of several months' duration. These experiments were conducted upon intact animals. The importance of a feasible implantation technique becomes apparent when it is considered that little or nothing is known concerning such fundamental questions as the functional possibilities of grafted pituitary tissue, the normal control of pituitary activity, and the cyclic nature of hypophyseal function—all involving as they do the crucial problem of the necessity or otherwise of nervous connections for normal physiological function. The technique, accordingly, has been applied to the problem of the activity of grafts in the hypophysectomized animal, with particular reference to the maintenance of gonadal function and to the interesting problem of the cyclic nature of pituitary activity. A consideration of the influence of such grafts upon the condition and functional activity of other glands of the endocrine system will be deferred for future discussion, the present communication being concerned only with certain phases of the pituitary-gonadal relationship.

EXPERIMENTAL PROCEDURE

Adult female guinea pigs, averaging between 400 and 500 grams, were hypophysectomized by the parapharyngeal approach. At the time of operation the anterior lobe tissue of a freshly killed female was bisected and implanted, one half in each anterior eye chamber, by a technique previously described (1). Animals were sacrificed at various intervals from 3 to 13 weeks after the operation, and ovaries, uteri, thyroids, adrenals and mammary glands were prepared for histological study. Mallory's connective tissue, Kingsley's polychromatic, as well as the routine Delafield's haematoxyl-eosin stains were used to differentiate cellular elements of the anterior pituitary. In every instance the sella was scraped and any fragments found were carefully removed and studied in serial section; only those cases in which no trace of anterior lobe

[1] Department of Physiology, Ohio State University.

tissue existed in the sella are included in this report. In several cases in which the animal was permitted to run for 8 weeks or more, a unilateral oöphorectomy

TABLE 1. CASE HISTORIES OF HYPOPHYSECTOMIZED-IMPLANTED ANIMALS.

No.	Experimental Period* days	Remarks: Gross Findings	Remarks: Histological Findings
1006	21	Uterus well developed	*Ovary:* medium-sized follicles; degenerating corpora lutea *Uterus:* glands and epithelium well maintained
1008	21	Vagina moist; uterus well developed	*Uterus:* numerous glands; epithelium well preserved
1004	22	Uterus well developed	*Ovary:* well maintained; various sized follicles with well-filled cavities and numerous mitotic figures *Uterus:* numerous convoluted glands; epithelium well preserved
1001	26	Uterus well developed	*Ovary:* numerous follicles; no corpora lutea *Uterus:* numerous convoluted glands; epithelium well maintained
1015	29	Uterus hyperemic, well developed. Right ovary, 45 mg. Left ovary, 38 mg.	*Ovary:* few medium-sized follicles; corpora lutea present *Uterus:* congested; numerous well-preserved glands; epithelium well outlined
1012	34	Uterus well developed	*Ovary:* numerous small follicles *Uterus:* numerous convoluted glands; epithelium well preserved
1007	52	Vagina nearly open; uterus well developed	*Ovary:* large well-formed follicles; numerous mitotic figures; follicular cavities well filled *Uterus:* numerous glands; epithelium well preserved
1016	56	Left ovary removed 26th day, 35 mg. Uterus pale at time of sacrifice. Right ovary, 27 mg.	*Ovary:* many large follicles *Uterus:* definite hypertrophy of muscularis; only slight increase in number of glands
1019	83	Right ovary removed 42nd day, 41 mg. At sacrifice uterus large and hyperemic. Left ovary, 40 mg. Constant estrus from 68th day	Same as above, with greater uterine hypertrophy
1018	93	Right ovary removed 16th day, 20 mg. At sacrifice uterus very large, hyperemic. Left ovary, 45 mg. Constant estrus from 53rd day	Same as above

* Time elapsed between date of hypophysectomy and transplantation and date of sacrifice.

was performed at an earlier period, one upper horn of the uterus being included in removal. These tissues were used for comparison with those removed at the time of sacrifice. Controls for our animals with an experimental period

of 1 month or less were obtained by sacrificing hypophysectomized unimplanted animals at the end of 3 weeks. For comparison with our cases of longer duration we have animals which were subjected to the same operative and implantation procedure as our experimental pigs, the implants in these instances, however, having failed to take. Table 1 gives the record of all our hypophysectomized animals in which the operation was complete.

In instances in which the implants proved successful the animals remained in excellent condition, gained in weight and were apparently in perfect health at the time of sacrifice. In animals sacrificed between the 21st and the 52nd day after operation the ovaries were in better condition than those of the unimplanted hypophysectomized controls, but the striking hypertrophy of the uterus characteristic of animals which were allowed to run for longer periods was not found until nearly 2 months had elapsed from the time of operation. In the animals with an experimental range of 56 days or longer a striking degree of hyperplasia appeared, the uterine horns were engorged and the ovaries were large and distinctly follicular. This follicular growth was not reflected by increase in ovarian weight but was readily apparent both grossly and microscopically. The histological appearance of ovaries, uterus, thyroids, adrenals and mammary glands of these animals was in striking contrast to the degenerate condition of these structures in animals in which the implants were unsuccessful. In the experimental cases 1018 and 1019 the vagina opened during the 53rd and 68th day, respectively, and remained open until the time of sacrifice.

HISTOLOGICAL FINDINGS

Anterior Pituitary. The implants were recovered from animals sacrificed from 21 to 93 days after hypophysectomy and transplantation (table 1, fig. 1). In every case here reported the implants were well maintained. In several cases showing fragmentation of the implant, a portion of anterior lobe tissue had become secondarily attached to the outer surface of the eyeball in the region of the lateral subconjunctival tissue (fig. 1, *5, 6*). For the most part the implanted tissue was recovered attached to the pupillary rim of the iris (fig. 1, *3, 4*).

All the gland tissue examined showed gland cells in the usual nest formations, bounded by fairly well-defined capillary sinuses which were more patent in the intra-ocular gland tissue. Occasional groups of blood cellular elements could be found in between and around the gland cells. A goodly scattering of mitotic figures could be seen in all fields examined. Differential staining (Mallory's triple connective tissue stain) of the implanted tissue showed a definite dominance of the basophilic elements. In tissues recovered after a 50-day period eosinophilic elements were rare and occasional chromophobic cells could be found interspersed between the basophiles. Both intra- and extra-ocular implants showed an increase in the compactness of the glandular tissue (fig. 1, *3-6*).

Ovary. Ovaries of the experimental animals all showed degenerating luteal bodies and, in later stages, only isolated groups of retrogressing luteal cells; in occasional regions there were small groups of atypical glanulosa cells. In tissue examined from later stages no well-formed luteal cells could be found at all.

A goodly number of different-sized follicles embedded in varying amounts of stroma could be seen in the fields studied.

Follicles in the ovaries recovered from animals 21 to 34 days after operation were medium sized, with many of them containing well-formed follicular cavities and others showing the aggregate vacuolation so indicative of the be-

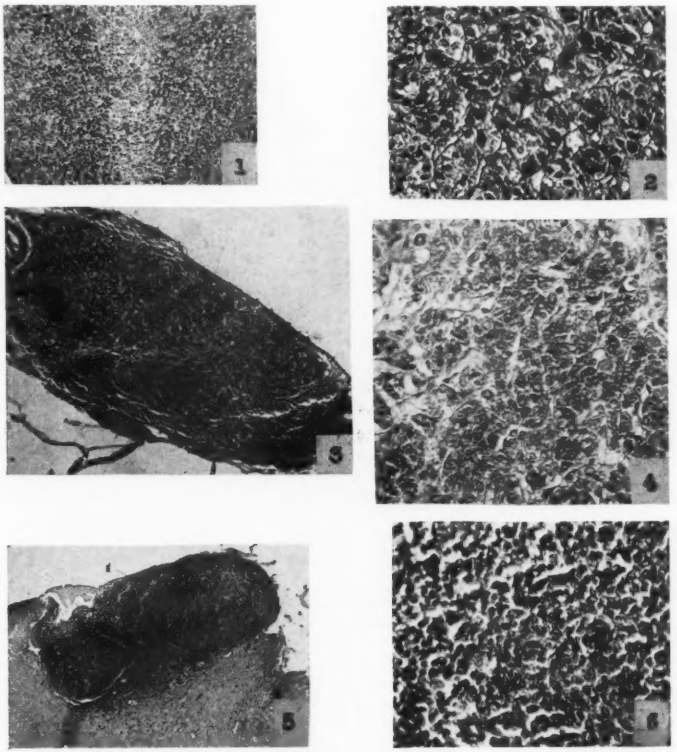


FIG. 1. Photomicrographs of anterior pituitary (haematoxylin-eosin). *1)* Anterior pituitary gland tissue from a normal, unoperated, mature female guinea pig showing general topography (× 48). *2)* Normal gland showing presence of different cell types arranged in nests surrounded by well defined sinusoids (× 240). *3)* Intra-ocular implant attached to the pupillary margin of the iris showing slight increase in compactness, 93 days (No. 1018) (× 48). *4)* Same as *3)*, under higher magnification, showing persistence of nesting formation with a definite dominance of the basophilic cells (× 240). *5)* Extra-ocular implant attached to the lateral subconjunctival tissue showing general topography and increase in compactness, 93 days (No. 1018) (× 48). *6)* Same as *5)*, under higher magnification, showing the dominance of the basophilic cell type with a most apparent relative absence of eosinophilic cells (× 240).

ginning of follicular cavity formation. In ovaries recovered from animals sacrificed after 52 days there were many fairly large-sized follicles containing clear, well-defined cavities filled with follicular fluid. The theca externa cells were clearly defined and merged into the area of the interna in which could be found numerous mitotic figures distributed among the large and slightly granular cells containing the usual vesicular nucleus with its clear chromatin network. The cortex of the ovaries contained many well-maintained primordial follicles of varying sizes. In no case was there evidence of ovulation (fig. 2, 7, 8).

The ovary from hypophysectomized-unimplanted animals showed the usual degenerative changes such as reduction in size, pyknosis of granulosa cells and general stromification of the gonad.

Uterus and Cervix. The uterus showed by far the most striking changes, reflected particularly by increase in size, which is of the order of 4 to 5 times that of unoperated controls (fig. 2, *9, 10;* fig. 3, *13, 14*). There was a pronounced thickening of both endometrium and myometrium. The epithelial cells lining the lumen of the uterus had become definitely more columnar and in places appeared to be stratified. Numerous mitotic figures could be seen among the epithelial cells of the uterine glands, which were well-developed and showed

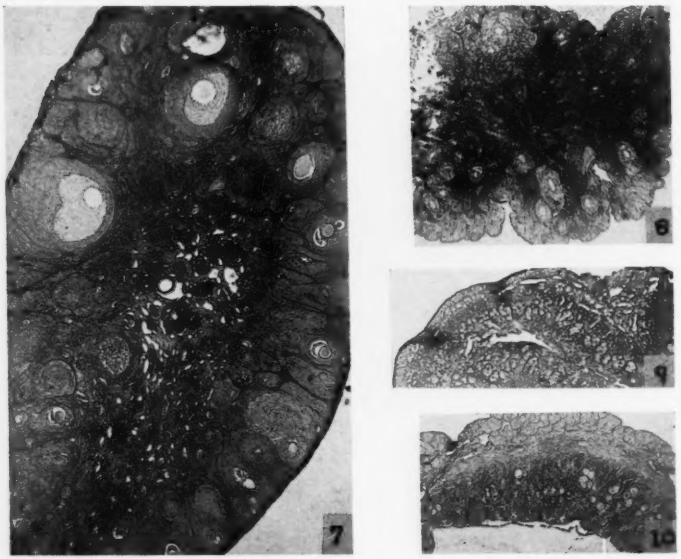


FIG. 2. Photomicrographs of the ovary and uterus (haematoxylin-eosin), (× 15). *7)* Ovary from unoperated mature animal. *8)* Ovary from hypophysectomized animal with no implant, 63 days postoperative, showing degenerative changes (control). *9)* Portion of uterus from 63 day post-operative control, previously figured, showing reduction in muscularis and disorganization of uterine glands. *10)* Portion of uterus from animal of *7)* showing the amount of muscularis and organization of uterine glands in unoperated mature animal.

some branching toward their basal tubular terminations. Many blood vessels could be seen all of which contained blood cellular elements. The myometrium showed an increase in muscle cell elements as well as a considerable amount of connective tissue arranged in septa between the bundles of smooth muscle cells.

The cervix revealed a condition similar to that to be found in the uterus, in that marked hypertrophic changes could be seen. The epithelium showed an increase in stratification with larger and more distended cells, and the cervical glands were definitely increased in size and number with many mitotic figures present. The uterus from hypophysectomized-unimplanted controls showed a definite decrease in the muscularis with disorganization of the uterine glands and hyalinization of the uterine marginal epithelium (fig. 2, *9*).

Mammary Glands. In view of conditions obtaining in the ovaries and uterus it is interesting to note the histological picture of the mammary glands. In all cases examined the glands showed an increase in the size of ducts and in some instances small alveoli could be found. The epithelium lining the ducts

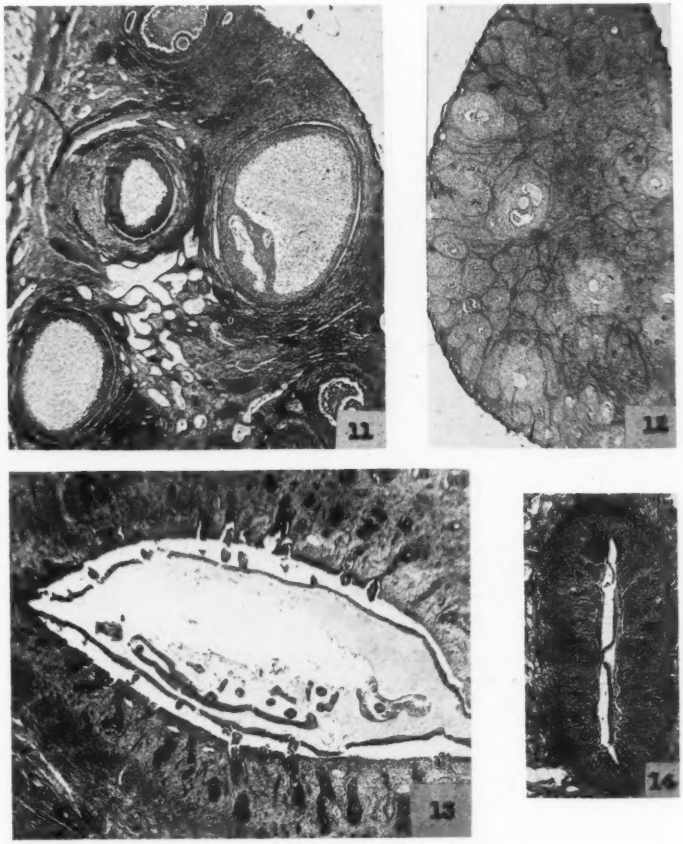


FIG. 3. Photomicrographs of the uterus and ovary from hypophysectomized, implanted animal, 1018. (× 15). *11)* Ovary recovered 93 days after hypophysectomy-implantation, showing increase in number of large follicles with well organized cavities and no apparent luteinization. *12)* Ovary recovered 16 days after operation. *13)* Cross-section of uterus recovered 93 days after operation, showing general growth, increase in size and number of uterine glands and thickening of the muscularis. *14)* Cross-section of uterus recovered 16 days after operation, showing relative size, distribution of mucosa muscularis and uterine glands.

showed an increase in height and many mitotic figures. The nipples especially in the more protracted cases were increased in height and were somewhat turgid.

General. The thyroids and adrenals examined all showed good maintenance. The adrenals especially were well differentiated both in the cortical and medullary regions.

DISCUSSION

Successful transplantation of anterior lobe tissue has been reported recently from several laboratories (1, 2, 3, 4, 5). With respect to the replacement capacity of grafts, some degree of testis repair and an increase in body size has been observed in 2 hypophysectomized rats carrying intra-ocular implants of tissue from newborn animals (4). Two cases, moreover, have been described (6) in which intratesticular grafts from sister mice maintained the testes of hypophysectomized males. The glands were supported at an apparently normal level and, in one instance, an ovary implanted in a testis was maintained as well, having been recovered as a 'good ovarian graft.' More recently, (7), a preliminary report has appeared in which it is stated that pituitary grafts in the empty sellae of hypophysectomized 28-day old rats induced a marked body growth and resulted in complete sexual development. This report is of particular interest in that females bearing either male or female pituitary grafts displayed cyclic activity and, in a number of instances, underwent complete reproductive cycles. It seems difficult to reconcile these findings with the reports from Witschi's laboratory (*vide infra*) or with results obtained in the present work. It is not inconceivable, moreover, that implantation on the original site of the gland would favor the re-establishment of normal neuro-vascular relations which would, essentially, defeat the purpose of transplantation. There is, however, no proof that such is the case and it is obvious that more work must be done before a complete explanation can be obtained.

One of the interesting observations in the present work is the fact that 2 of the experimental animals displayed after a time a condition of constant estrus. Examination of the serially-sectioned ovaries revealed an exclusively follicular condition with no evidence of formed corpora lutea. The follicular stimulation was not a maximal one, however, in that neither fully mature nor cystic follicles were present and the follicles gave the appearance of having been maintained at a slightly subnormal level. It would seem that a generation of follicles was maintained for a period, after which these became atretic and were replaced in turn by a new generation, since follicles in varying stages of growth and of degeneration could be found. The absence of well-defined corpora lutea or even of easily discernible groups of luteal cells under these circumstances is rather striking. Unilateral oöphorectomy in all probability may have caused some degree of hypertrophy of the remaining ovary but this fact in itself does not account for the marked changes in the ovary and uterus which were obtained in our long-term cases, since it did not occur at all if a unilateral oöphorectomy was performed in an animal which was incompletely hypophysectomized, even though it was implanted.

The absence of well-defined luteal tissue deserves further consideration. In the studies reported by Witschi and his students (8, 9, 10, 11) on parabiotic rats, the ovaries were stimulated for long periods by FSH in high concentration; ova, moreover, were never released nor were corpora lutea formed. Constant estrus appeared after a time in females in parabiotic union with castrates. Follicles maintained over long periods of time eventually degenerated, a singular characteristic of such atresia being the entire absence of luteinization. The

necessity for LH not only for luteinization but for the final stages of follicular maturation and for ovulation as well has received emphasis (13) in experiments which indicate that LH acts in an 'augmentation' or synergistic capacity to FSH in producing rupture of the follicle. The fact that ovaries in the present experiments were maintained at a slightly subnormal level in the absence of LH would seem to support this view.

A somewhat similar situation, although unique in some respects, has been reported (11) in experiments in which newborn female rats were implanted with litter-mate testes. Hypophyses of rats so treated became 'male' in character, secreting only FSH and secreting that constantly. No LH was released, although estrin was present in some instances in marked concentration. Ovulation did not occur spontaneously and follicular atresia was unaccompanied by luteinization, although both responses could be brought about in the experimental animal by the administration of suitable luteinizing extracts. Such animals were characterized as being in 'subnormal estrus,' that is, vaginal smears revealed persistent estrus with the uteri but slightly maintained and the ovarian follicles did not attain full mature size. In the writers' opinion, this lack of follicular development and of complete uterine stimulation is a consequence of the absence of LH, a condition obviously obtaining in the case of the animals described in the present report.

The absence of cyclic activity in the normal immature female may perhaps be attributed to a deficiency in LH as well. In the prepubertal rat, for example, it is believed (14) that only subminimal amounts of LH are secreted, in view of the circumstance that frequently the administration of FSH did not result in luteinization and, moreover, by the fact that FSH action apparently was confined to its capacity for increasing ovarian weight.

In our experimental animals there is every indication that an excessive quantity of estrin was produced, as evidenced by the condition of the uteri and by the continuous estrual smears. This circumstance, combined with the absence of ovulation and of luteinization, even in atretic follicles, suggests rather a unique endocrine condition. It is believed that the hypophyseal grafts are functional, but only to the extent of releasing follicle-stimulating hormone. The homoplastic anterior pituitary grafts in the hypophysectomized guinea pig, therefore, appears to have lost its cyclic character, at least for the time limits employed in the present experiments. A constant release of FSH prevails, sufficient to stimulate a marked degree of follicular development. The absence of cyclic activity may be attributed to the failure of periodic release of luteinizing hormone, if one accepts the concept of a separate and specific principle, which in the normal animal (13) apparently augments the action of FSH in inducing periodic maturation, ovulation and the resultant luteinization of pseudopregnancy, and upon the timely action of which, indeed, a theory for the mechanism of the estrous cycle is based (15). The question of paramount importance seems to be that of the role played by nervous connections in this release. Evidence from other laboratories would indicate that the ultimate condition of constant estrus may be the result of an equilibrium established, in the absence of LH, between estrin and the FSH release of the anterior lobe; in the present

experiments essentially the same type of equilibrium appears—the absence of LH, initially at least, presumably being due in this case to the severance of the neural pathways for stimuli to the hypophysis. Recent evidence (16, 17) may be advanced in support of the theory that loss of normal nervous connections prevents the release of LH, which indicates that nervous control of the pituitary is a prime factor in the secretion of luteinizing hormone, at least in the rabbit. It appears probable that in this form the excitation resulting in LH secretion passes through central nervous channels. Furthermore, observations in the rat (18) indicate that in this form severance of the pituitary stalk results in decidedly atypical cycles.

It would seem that the final criterion as to whether, in an animal which normally displays cycles, periodic release of LH is dependent upon nervous control of the pituitary, lies in the answer to the question of whether or not the gland will retain its cyclic properties when severed from its nervous connections and transplanted to a new site. The present work, in which the original gland is completely removed and in which anterior lobe tissue from another individual is shown to have the capacity for replacing it so far as maintenance of the gonads is concerned, would appear to furnish a conclusive answer. The cyclic phenomena associated with the pituitary-gonadal relation in the normal animal are completely absent.

In regard to the implantation of anterior pituitary tissue, it is interesting to note that the lateral subconjunctival tissues evidently affords a good site for this tissue and in so doing may obviate the necessity of resorting to a corneal-scleral incision, a somewhat delicate procedure on small animals. Transplanted anterior lobe tissue recovered in the course of these experiments appeared noteworthy chiefly because of a pronounced basophilia. This cellular condition obtaining during estrous is similar to that reported for the albino rat (19) and is to some extent in accord with the more recent description of the histological nature of the anterior pituitary in the guinea pig during the various phases of its estrous cycle (20). It is, of course, hazardous to speculate upon the significance of this circumstance although, in view of the situation in the rat (21), it is tempting to assign the FSH activity to the abundance of the basophilic cell types. A more detailed consideration of the cytological features of the pituitary grafts, including those of other endocrine glands maintained in the hypophysectomized-transplanted animal, will form the basis of a subsequent communication.

SUMMARY

Intra-ocular implants attached to the pupillary margin of the iris, and extra-ocular implants in the lateral subconjunctival tissues became dominantly basophilic and retained their gonadotropic capacity in completely hypophysectomized guinea pigs. The ovaries became progressively more follicular until after approximately 2 months a condition of constant estrous supervened. This condition is characterized by a continual estrous vaginal smear, by a markedly hypertrophied uterus, and by well proliferated mammary glands. The ovarian effect of such implants was exclusively a follicle-stimulating one; the ovaries contained no well-defined luteal bodies, and the endometrial pattern, moreover,

was typically that brought about by estrin influence. The complete absence of luteinizing hormone was further attested by the absence of fully mature follicles and of ovulation.

On the basis of these observations, the conclusions are advanced that the hypophyseal grafts were functional in maintaining the ovaries in the follicular phase and that the guinea pig pituitary when transplanted to a site removed from its normal anatomical position lost its cyclic character. This cyclic failure, in all probability, was due primarily to the severance and non-regeneration of its normal neural and vascular connections.

We wish to express our appreciation to Professor G. N. Papanicolau, of the Cornell University Medical College, for his examination of and suggestions concerning histological preparations; to Mr. Stanley Kahn for the photomicrographs; and to Mr. John E. Woolard for the histological preparations.

REFERENCES

1. Haterius, H. O., M. Schweizer and H. A. Charipper: Endocrinology **19:** 673. 1935.
2. Hohlweg, W., and K. Junkmann: Klin. Wchnschr. **11:** 321. 1932.
3. Gardner, W. U., and R. T. Hill: Proc. Soc. Exper. Biol. and Med. **32:** 1382. 1935.
4. May, R. M.: Compt. rend. Soc. de biol. **120:** 867. 1935.
5. Buxton, C. L.: Anat. Rec. **64:** 277. 1936.
6. Hill, R. T., and W. U. Gardner: Proc. Soc. Exper. Biol. & Med. **34:** 78. 1936.
7. Greep, R. O.: Proc. Soc. Exper. Biol. & Med. **34:** 754. 1936.
8. Witschi, E., and W. T. Levine: Proc. Soc. Exper. Biol. & Med. **32:** 101. 1934.
9. Du Shane, G. P., W. T. Levine, C. A. Pfeiffer and E. Witschi: Proc. Soc. Exper. Biol. & Med. **33:** 339. 1935.
10. Witschi, E., and C. A. Pfeiffer: Anat. Rec. **64:** 85. 1935.
11. Pfeiffer, C. A.: Am. J. Anat. **58:** 195. 1936.
12. Hellbaum, A. E.: Proc. Soc. Exper. Biol. & Med. **30:** 641. 1933.
13. Fevold, H. L., F. L. Hisaw and R. Greep: Am. J. Physiol. **114:** 508. 1936.
14. Meyer, R. K., S. L. Leonard, F. L. Hisaw and S. H. Martin: Endocrinology **16:** 655. 1932.
15. Hisaw, F. L., H. L. Fevold, M. A. Foster and A. E. Hellbaum: Anat. Rec. **60:** 52. 1934.
16. Brooks, C. M.: Am. J. Physiol. **113:** 180. 1935.
17. Marshall, F. H. A., and E. B. Verney: J. Physiol. **86:** 327. 1936.
18. Richter, C. P.: Am. J. Physiol. **106:** 80. 1936.
19. Charipper, H. A., and H. O. Haterius: Anat. Rec. **54:** 15. 1932.
20. Chadwick, C. S.: Am. J. Anat. In press, 1936.
21. Severinghaus, A. E.: Anat. Rec. **60:** 43. 1934.

THE IMPORTANCE OF CORTICOADRENAL REGULATION OF POTASSIUM METABOLISM[1]

R. L. ZWEMER AND R. TRUSZKOWSKI
Department of Anatomy, College of Physicians and Surgeons, Columbia University
NEW YORK CITY

Corticoadrenal insufficiency has been shown by extirpation experiments and replacement therapy to be responsible for the sequence of events grouped under the general term of adrenal insufficiency, but there are many theories and little agreement as to what action of the adrenal cortex is essential for the well-being of the body as a whole.

The pathologic anatomy of cases of experimental adrenal insufficiency terminating without supervention of infections showed certain histologic changes of which the most definite referred to the kidneys and lymphoid structures (1). None of these changes were, however, of sufficient gravity in themselves to be held responsible for the death of the animals. The morphology of the adrenal glands after experimental procedures and in pathologic conditions not directly associated with adrenal insufficiency showed that the adrenal cortex was most depleted in conditions involving excessive catabolic activity or cell breakdown (2). The structural changes seemed to depend mainly on the degree and duration of the condition and not on the nature of the stimulus or toxin.

A comprehensive study of the blood chemistry of adrenalectomized cats (3, 4) indicated that a fall in alkali reserve, sodium and glucose with an increase in non-protein nitrogen and potassium commenced early in the syndrome and varied differently during its development.

In the absence of anatomical criteria the changes in blood chemistry have been the subjects of numerous studies having as their object the explanation of the observed symptoms and the establishment of procedures for the diagnosis of adrenal insufficiency. These attempts have not, on the whole, been successful since the values found for glucose, non-protein nitrogen and sodium are not incompatible with survival and have each been reproduced experimentally without producing the symptoms of adrenal insufficiency. With respect to potassium, however, the problem remained practically untouched except for unsubstantiated views that the potassium levels might be high enough to produce toxic effects and some data as to the serum-potassium level in adrenal insufficiency in cats (4, 5, 6, 7, 8), dogs (9) and humans (10).

In view of the known toxicity of the potassium ion, its ready availability within the cells of the organism and its normally low concentration in plasma and other body fluids (11), further investigation of corticoadrenal involve-

[1] Read before the 20th Annual Meeting of the Association for the Study of Internal Secretions, Kansas City, Mo., May 11, 1936.

ment in potassium metabolism was desirable. The following points were specifically dealt with. *A*) The changes in blood and muscle potassium in adrenalectomized animals on an unvaried diet. *B*) The clinical effects and blood-potassium levels in normal animals given injections of potassium. *C*) The susceptibility of adrenalectomized animals to potassium feeding or injection. *D*) The protection of normal animals from potassium poisoning with adrenal cortex extract.

MATERIALS AND METHODS

Approximately 35 cats, 25 rats, 75 mice and 15 guinea pigs were used in these experiments.

The potassium content of blood, muscle and food was determined by a method described elsewhere (8). Adrenal cortex extract was made from fresh

TABLE 1. DAILY BLOOD POTASSIUM IN ADRENAL INSUFFICIENCY. VALUES IN MG. PER CENT K IN PLASMA, SERUM, OR WHOLE BLOOD.[1]

	Normal Values	Days after adrenalectomy												
		1	2	3	4	5	6	7	8	9	10	11	12	12+
Maximum	23.1	27.3	30.2	42.5	39.2	44.2	44.4	31.1	44.4	39.0	34.2	31.6	37.5	46.1
Minimum	14.0	22.2	21.4	31.2	20.5	21.7	22.4	21.0	11.8	11.1	17.1	17.5	22.7	22.1
Average[2]	19.3	24.9	26.7	36.4	30.0	30.3	27.9	24.5	29.4	25.1	26.2	24.6	27.7	34.2
No. of cats	19	3	5	6	6	7	9	3	6	5	5	3	3	11

[1] In the cat the K content of cells and plasma is the same and both rise or fall together.
[2] Complete data on individual cases are given elsewhere (8).

beef adrenal glands according to a method which has been briefly outlined (12), and is a shortened form of an earlier procedure (13).

EXPERIMENTAL RESULTS

A. Blood and muscle potassium in adrenalectomized cats. In general there is a rise in blood potassium after adrenal removal. Since, however, the level may be normal or even sub-normal during certain phases of the syndrome, the values at any one time are not always a criterion of the degree of insufficiency present. This is best illustrated by graphs of 2 individual cases (fig. 1). One animal (curve 1) had a rise in blood potassium which continued until death. Another cat (curve 2) had the usual 3rd or 4th-day rise while still eating and active, then, coincident with a voluntary marked reduction of its food intake and muscular activity, had a fall in blood potassium to almost normal limits. As will be shown later, there is in these animals an incipient but definite inability to handle even small additional amounts of potassium.

Both skeletal and cardiac muscle of adrenal-insufficient cats have lower potassium contents per 100 grams wet weight than normal (table 2), but due to an increase in water content the dry-weight potassium is greater than normal. It is difficult as yet to interpret these changes, but they may be associated with a number of conditions which are known to affect muscle K and are present in adrenal insufficiency.

TABLE 2. MUSCLE POTASSIUM AFTER ADRENALECTOMY.

Per cent K in muscle	Normal[1]		Adrenalectomized Cats	
	Skeletal	Cardiac	Skeletal (6)	Cardiac (3)
Wet range	0.450–0.500	0.365–0.368	0.375–0.457	0.211–0.309
Average	0.480	0.366	0.415	0.275
Dry range	1.689–1.778		1.811–1.981	1.030–1.405
Average	1.733	1.300	1.900	1.277
Dry content	27.2–28.2	26–29	20.2–24.1	20.5–22.0

[1] As given by Leulier et al (14) plus a few of our own determinations.

B. Injections of potassium salts into normal animals. These were given to see whether increased potassium could be responsible for the syndrome of adrenal insufficiency. Injections given intravenously or intracardially led within a few minutes to death with convulsions, if large amounts were administered, whereas smaller injections produced no observable symptoms. On the other hand, intraperitoneal injections were followed by symptoms which lasted for half an hour or more, if death did not intervene. Some of the recovered animals

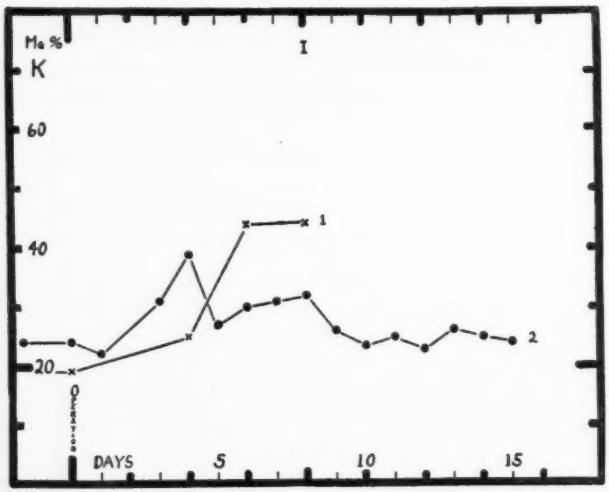


Fig. 1. Blood potassium after adrenalectomy. Curve 1. Showing potassium rise after adrenalectomy in the cat with progressive development of the syndrome, no remission of symptoms and death on the 7th day. Curve 2. Similar initial rise, but later a fall concordant with a marked decrease in food intake and activity. Death occurred on the 15th day.

when used a day or so later showed a diminished tolerance to a sub-lethal second dose, and this led to experiments on repeated small daily injections. The lethal single injection dose of KCl for various species is given in table 3; it indicates that the dose per 100 gm. body weight is only 15 to 20 per cent of the amount present in 100 gm. of skeletal muscle or human red blood cells, yet the plasma-K content would be increased approximately 90 fold if no

potassium were eliminated by the kidneys or taken up by the body cells. Determination of blood potassium in the normal cat after KCl injection shows that this of course is not the case.

The plasma values represent equilibria between potassium being absorbed from the peritoneal cavity and that being eliminated from the plasma. In an animal with adequate adrenal function the injection of 200 mg. per kg. body weight caused a rise which reached its peak of 33 mg. per cent after about an hour and was back to normal in 2 hours (fig. 2, curve 3). No striking symptoms accompany such a transient rise. If a second injection is given 1 hour after the first, there is a cumulative rise of blood potassium to a level having a

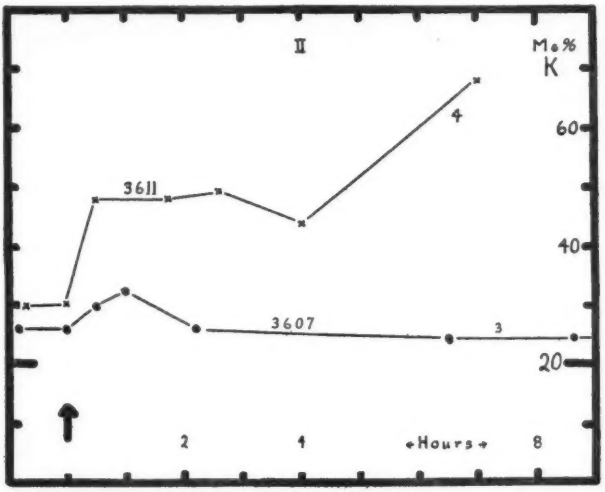


Fig. 2. "Potassium Tolerance Curves". Curve 3. Effect of injection of 200 mg. KCl per kg. body wt. in an animal with one adrenal; note rapid return to preinjection level. Curve 4. Same amount per kg. given to an animal 6 days after removal of both adrenals resulted in death in 7 hours.

definite effect on circulation and muscular activity. In addition, the ability to lower plasma potassium is interfered with and the level remains high for some hours. This rise is even more persistent if the animal has been subjected to previous daily injections of potassium (fig. 3, curve 5). The histology of the adrenal glands of animals treated with K indicates that corticoadrenal depletion has occurred.

Detailed studies on experimental hyperpotassemia will be published elsewhere, but we can here state that observable symptoms and circulatory changes typical of the various manifestations of adrenal insufficiency and of shock can be reproduced in normal animals by appropriately spaced injections of solutions of potassium salts.

C. The susceptibility of adrenalectomized animals to potassium in their diet or to injections of potassium salts. In the experience of this laboratory, 90 per cent of rats (4 strains used) will survive adrenalectomy (15) if kept on a well-balanced diet and a selection of food is permitted. By giving a diet

which had a high potassium content regardless of selection (i.e., adding KCl to foods low in potassium such as white bread) the mortality rate was greatly increased, approaching those reported by a number of investigators since 1930. The short survival was in some measure due to starvation because of a disinclination for food of high potassium content (0.5 gm.%K). Recovery could be effected by giving low potassium food (0.1 gm.%K) which was readily eaten.

Some adrenalectomized cats were also given food of high potassium content a day or two after their operation, when still vigorous and with good appetites. They became nauseated after a meal or two and refused to eat any

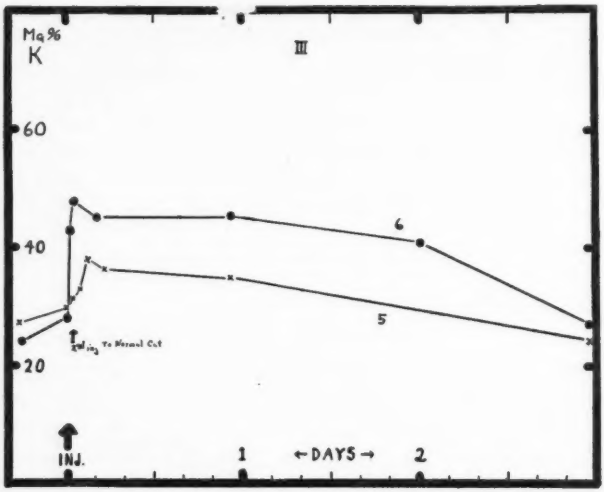


Fig. 3. Prolonged hyperpotassemia. Curve 5. A normal cat given 2 injections of 200 mg. KCl/kg. one hour apart had a rather prolonged rise in blood K. It had previously had a number of injections of potassium salts. Curve 6. Two days after adrenalectomy a single injection of 150 mg. KCl/kg. proved almost fatal, but partial recovery followed a gradual decrease in blood K.

more, although identical food was being eaten with relish by normal cats. The replacement of the highly potassic food with a diet of washed salmon and milk restored the appetite in a few cases. As we were interested in quantitative effects of potassium administration and felt that forced feeding would in itself shorten the survival time, we next tried intraperitoneal injection of known amounts of potassium salts into adrenalectomized cats. The blood potassium was subsequently determined at intervals and correlated with the clinical manifestations. Two typical examples are presented in graphic form.

The injection of 200 mg.KCl/kg. into a cat on the 6th day after adrenalectomy produced striking results. The entire sequence of events in the syndrome, which would ordinarily have taken days to develop, occurred in 7 hours. Anorexia, gradually increasing asthenia, progressive diminution of peripheral circulation, profound shock, and terminal convulsions were observed. The blood potassium rose rapidly, then remained fairly constant at a

TABLE 3. LETHAL INTRAPERITONEAL KCL.

Species	Approx. wt. used gm.	Lethal Dose as cc. of 10% KCl	Grams of K per 100 gm. body weight
Mouse	20	0.2	0.052
Rat	200	3.0	0.079
Guinea pig	300	4.0	0.066
Cat	3000	30.0	0.052

high level for some time. A slight drop was associated with a temporary remission of symptoms, followed by a terminal rise to a higher level (fig. 2, curve 4).

A slightly smaller amount (150 mg.KCl/kg.) appeared to be just within the limits of tolerance of another operated animal (fig. 3, curve 6). At the peak of the rise there was profound muscular weakness and circulatory disturbances. Some cats died at this stage, when, in picking them up by the scruff of the neck, the already inadequate circulation to the head was still further diminished and convulsions ensued. The latter were probably a consequence of cerebral anemia or stagnation. Concomitant with the gradual lowering of blood potassium there was a return of skeletal muscular activity and an improvement in the circulation. Recovery to the pre-injection level did not occur, however, and the cat survived the test by only a day or two.

The expected survival period was definitely shortened in all cases by amounts of potassium that were readily disposed of by normal animals, being one-fifth or one-sixth of the lethal dose. This increased susceptibility to potassium after adrenalectomy led to experiments in which an attempt was made to protect normal animals from lethal potassium injection by treatment with adrenal cortex extract.

D. The effect of adrenal cortex extract on potassium poisoning in guinea pigs and mice. Two illustrations of the effect of cortin on potassium poisoning in normal animals are given in tabular form. One group of 3 normal guinea pigs survived less than half an hour after an injection of 4 cc. of 10 per cent KCl, whereas the same amount of potassium following administration of adrenal cortex extract did not prove uniformly fatal to three others (table 4).

Daily injection of three-fourths the lethal dose of potassium killed all 4

TABLE 4. EFFECT OF CORTIN AND SINGLE LETHAL DOSE OF POTASSIUM IN GUINEA PIGS.

Animal No.	Weight gm.	Amount of Cortin cc.	Hours before KCl	Amount of 10% KCl injected cc.	Survival time minutes
K 12	282	none	—	4	17
K 7	300	none	—	4	23
K 13	303	none	—	4	27
K 9	340	2	3	4	41
K 11	325	3	3	4	7 hours
K 10	268	3	3	4	Lived

control mice in 6 days. At that time 3 of 5 cortin-treated animals were still alive and vigorous (table 5). Injections were continued on these 3 for one week more, when they died, after receiving twice as much KCl as the controls. Their survival was perhaps shortened by the development of local lesions at the sites of repeated injections. Other groups of mice, used to test variations in time, the amount of KCl injected and extract potency, confirm the protective effect, but are not otherwise pertinent to this report.

E. Effect of adrenal cortex extract on the blood potassium of normal cats. The injection of 1 cc. of cortin per kg. of body weight lowers the blood potassium of normal animals 20 per cent within one hour of injection, the lower level being maintained for some hours. This is compatible with the

TABLE 5. EFFECT ON MICE OF REPEATED INJECTIONS OF 15 MG. KCl WITH AND WITHOUT ADRENAL CORTEX EXTRACT.

Animal No.	Weight gm.	No. of injections	Total KCl mg.	Survival time
		15 mg. KCl in 0.6 cc. H_2O		
M 30	19	1	15	14 min.[2]
M 27	21	3	45	3 days
M 22	20	5	75	6 days
M 24	20	5	75	6 days
		15 mg. KCl in 0.15 cc. H_2O + 0.45 cc. cortin		
M 28	21	1	15	12 min.[2]
M 26	21	3	45	3 days
M 21	20	6	90	alive[1]
M 23	21	6	90	alive[1]
M 25	18	6	90	alive[1]

[1] Injections were continued on these 3 mice after the death of the controls; they finally died after receiving 150 mg. of KCl in 10 injections over 13 days.
[2] About 20 per cent of the mice used for repeat injections failed to survive the initial injection of ¾ m.l.d.

observation of Zwemer and Sullivan (4) that adrenalectomized cats had a 50 per cent decrease in serum-K level after injection of potent adrenal cortex extract. This effect may prove to be as important for the standardization of cortin as the blood sugar decrease is for insulin. The plasma-K fall would seem to be more specific than the rise in CO_2 capacity previously suggested as a test (3), but further research may show a significant relationship between the two effects of cortin on normal blood chemistry.

DISCUSSION

Even a brief review of the literature concerned with the physiological and toxic effects of potassium would convince the most skeptical of its importance in bodily economy. Yet there is little exact information as to what blood-potassium levels adversely affect the actions of skeletal muscle, nerve, heart and viscera, nor what conditions favor potassium retention in the blood or loss from cells to the detriment of the organism as a whole.

The results of analyses presented above show that in adrenal insufficiency a normal intake of potassium cannot be properly handled. On a constant diet relatively high in potassium, anorexia occurs when the plasma-K reaches 30 mg. per 100 cc. The subsequent course of events depends to a large extent on the intelligence of the individual and the availability of a choice of foods. If only high potassium food is offered one may take too much with a rapidly fatal outcome, another will curtail its intake to starvation level, postponing death for a while. The selection of low-potassium food by adrenal insufficient animals is well illustrated by the fact that 90 per cent of rats survived adrenalectomy if they were given a choice of foods (15), whereas in the present experiments less than 50 per cent survived if only a high-K diet was available, although the other conditions of the experiment remained the same. The relation of diet to the survival of adrenalectomized rats is shown in the work of Cleghorn et al (16) in which the importance of a bread diet for survival is stressed. We analysed food of the type with which they obtained only a short survival and found a K content of approximately 0.7 per cent, whereas bread has about 0.1 per cent. Their experiments with adrenal cortex extracts incidentally illustrate the protective action of cortin against high-K diets in adrenalectomized animals. The toxic action of potassium in the food of adrenalectomized dogs (17) and in patients with Addison's disease (18) has also been reported.

Normal animals are protected from too large amounts of potassium by mouth by the onset of anorexia and even of vomiting. The m.l.d. by intraperitoneal injection varies with the species, being 1.5 times as much for the rat as for the cat or mouse. More than 1 m.l.d. has been given by repeated injection of sub-lethal amounts but there is a cumulative effect as shown by observation of symptoms and 'potassium tolerance curves'. The latter afford a quantitive method of measuring ability to deal with potassium, after parenteral or peroral administration of definite amounts per unit body weight.

In support of a positive action of the adrenal cortex on potassium, the lowering of blood K in adrenalectomized cats by cortin injection has been previously reported (4). We now find that plasma-K is lowered and excess K protected against in normal animals.

The relation of the symptoms of potassium poisoning to the known facts of adrenal insufficiency would require a monograph to discuss adequately, but the syndromes are remarkably similar. An explanation of the variations of the symptoms found by different investigators lies in the many points at which K can and does act. These depend on the acuteness of the blood-potassium rise, the intelligence of the individual or animal in meeting the situation and on individual differences in the sensitivity of different organs or systems, present before the potassium changes occurred.

Certain facts are of interest in view of the theories of corticoadrenal insufficiency now current. Potassium injection lowers blood sugar (19) and K is in other ways associated with carbohydrate metabolism (20, 21). The reciprocal relation of sodium and potassium is well known, in that each when administered brings about elimination by diuresis of the other, and also that

sodium protects against larger amounts of K in the normal diet than could otherwise be consumed (22). In uremia (with high non-protein nitrogen) it has been found that potassium is an important toxic factor (23). Administration of K salts brings about a lowering of the CO_2 combining power of the blood and acidosis. This in turn favors further release of K from the cells (24). The known effects of K on the heart and circulation in general (11) are sufficient to account for low blood pressure (25), peripheral stasis with consequent dehydration of the blood, and reduction of volume through capillary stasis and plasma loss. Muscles and nerves are also affected by alterations of the plasma-K content. Although in their case the relative cell:plasma-K ratio may prove to be of greater importance than the absolute plasma-K values, the evidence is still meagre. The ready fatigability of corticoadrenal insufficiency may be related to the potassium loss from muscle, since normal muscle has been shown to lose potassium, while gaining sodium chloride and water if experimentally stimulated to exhaustion (26). On the other hand, a reduction in blood supply (27) and a decrease in pH of the surrounding fluid (28) also favor loss of potassium from muscle cells. The susceptibility of adrenalectomized animals to histamine might also be related to the increase in blood K following histamine injection (29).

Discussion of adrenal-potassium involvement in toxic, traumatic and allergic conditions which are known to affect corticoadrenal morphology, are benefited by cortin therapy, or in which there is evidence for a potassium shift from cells to extracellular body fluids, must await further experimental work. Nevertheless, many papers in the older literature acquire new significance in considering potassium in their interpretation.

It is not yet possible to state in what way the adrenal cortex regulates potassium metabolism. In general, an increase in plasma potassium is due to its entry into the blood stream from the alimentary tract or from the cells, a decrease to elimination by the kidneys (and possibly the intestine) or fixation within cells. Therefore the constancy of the relatively low plasma level depends on the resorption and excretion of potassium by the body as a whole and by its individual cells. Each of these processes involves the differential permeability of living cell membranes toward potassium (with high concentration inside and low concentration outside the cell) and marked changes in this differential permeability are known to be fatal for the organism. Since corticoadrenal regulation of potassium metabolism may be directly related to differential permeability it would appear to be of general importance.

SUMMARY

Using a method for determining potassium in smaller amounts of blood or tissue than has heretofore been practicable, the following results have been obtained, using approximately 35 cats, 25 rats, 75 mice and 15 guinea pigs.

There are definite changes in blood and muscle potassium of adrenalectomized animals, which can be correlated with the observed symptoms. The various manifestations of the syndrome of adrenal insufficiency can be reproduced in normal animals by injection of solutions of K salts in amounts which

will raise and maintain the plasma-K at the levels found in adrenalectomized animals during similar phases of the syndrome.

Adrenalectomized animals cannot deal adequately with certain definite amounts of potassium given in food or by injection, although much greater amounts are easily disposed of by normal animals. Potassium tolerance as a quantitative measure of adrenal insufficiency should, however, be judged by frequent determinations of the blood-K level after K administration.

Injection of adrenal cortex extract (Zwemer) lowers blood K of normal cats and will protect normal mice and guinea pigs to some extent from otherwise fatal amounts of potassium administered intraperitoneally.

The importance of adrenal cortex involvement in regulating potassium metabolism is briefly discussed.

REFERENCES

1. MacMahon, H. E., and R. L. Zwemer: Am. J. Path. **5:** 491. 1929.
2. Zwemer, R. L.: Am. J. Path. **12:** 107. 1936.
3. Zwemer, R. L., and R. C. Sullivan: Proc. Soc. Exper. Biol. & Med. **28:** 723. 1931.
4. Zwemer, R. L., and R. C. Sullivan: Endocrinology **18:** 97. 1934.
5. Bauman, E., and S. Kurland: J. Biol. Chem. **71:** 281. 1926.
6. Urechia, C. I., G. Benetato and M. Retezeanu: Compt. rend. Soc. de biol. **119:** 439. 1935.
7. Zwemer, R. L., and R. Truszkowski: Science **83:** 558. 1936.
8. Truszkowski, R., and R. L. Zwemer: Biochem. J. In Press. 1936.
9. Hastings, A. B., and E. L. Compere: Proc. Soc. Exper. Biol. & Med. **28:** 376. 1931.
10. Marañon, G., J. A. Collazo, J. Barbudo and I. Torres: Arch. de med., cir. y especialid. **37:** 893. 1934.
11. Webster, R. W., and W. A. Brennan: Potassium and Tartrates. The Commonwealth Press, Chicago. 1927.
12. Zwemer, R. L., and C. W. Jungeblut: Proc. Soc. Exper. Biol. & Med. **32:** 1583. 1935.
13. Zwemer, R. L., F. J. Agate, Jr. and H. A. Schroeder: Proc. Soc. Exper. Biol. & Med. **28:** 721. 1931.
14. Leulier, A., A. Bernard and G. Béruard: Compt. rend. Soc. de biol. **114:** 58. 1933.
15. Agate, F. J., Jr., and R. L. Zwemer: Am. J. Physiol. **111:** 1. 1935.
16. Cleghorn, R. A., S. M. M. Cleghorn, M. G. Forster and G. A. McVicar: J. Physiol. **86:** 229. 1936.
17. Allers, W. D., H. W. Nilson and E. C. Kendall: Proc. Staff Meetings Mayo Clinic **11:** 283. 1936.
18. Wilder, R. M., A. M. Snell, E. J. Kepler, E. H. Rynearson, M. Adams and E. C. Kendall: Proc. Staff Meet., Mayo Clin. **11:** 273. 1936.
19. Kylin, E., and A. Engel: Klin. Wchnschr. **4:** 653. 1925.
20. Semler, R.: Klin. Wchnschr. **4:** 697. 1925.
21. Meyers, V. C., and G. Mangun: J. Biol. Chem. **114:** lxxv. 1936.
22. Bunge, G. von: Lehrb. d. physiol. u. pathol. Chem., Vogel, Leipzig, 1894.
23. Feltz and Ritter: Rev. méd. de l'est. **12:** 231. 1880. **13:** 14. 1881.
24. Fenn, W. O., and D. M. Cobb: J. Gen. Physiol. **17:** 629. 1934. Am. J. Physiol. **112:** 41. 1935.
25. Kleeberg, F.: Am. Med. **6:** 622. 1903.
26. Fenn, W. O.: Am. J. Physiol. **116:** 47. 1936.
27. Baetjer, A. M.: Am. J. Physiol. **112:** 139; 147. 1935.
28. Netter, H.: Pflüger's Arch. f. d. ges. Physiol. **220:** 107. 1928.
29. Thaler, J. I.: Proc. Soc. Exper. Biol. & Med. **33:** 368. 1935.

INCREASED CALCIUM APPETITE OF PARATHYROIDECTOMIZED RATS

CURT P. RICHTER AND JOHN FULTON ECKERT
Psychobiological Laboratory, Phipps Clinic, Johns Hopkins Hospital
BALTIMORE, MARYLAND

It was shown in a previous paper that the increased salt need of adrenalectomized rats is associated with a definitely increased appetite for salt, and that by means of this increased appetite such rats keep themselves alive and free from symptoms of adrenal insufficiency (1). With this fact as a basis it becomes of interest to know whether appetite is generally a guide to the needs created by glandular deficiencies. Inasmuch as the calcium need of parathyroidectomized rates is well established (2), a study was made of the level of calcium appetite in these animals before and after removal of the parathyroid glands.

METHODS

Eighteen rats were used in these experiments varying in age from 44 to 84 days at the time of parathyroidectomy. They were kept in individual cages containing a food-cup and 2 inverted graduated bottles, one filled with tap water, the other with a 2.4 per cent calcium lactate solution.

It was desirable to give the animals a diet as free of calcium as possible, so that their calcium intake would depend on their own voluntary ingestion of the calcium lactate solution. For this reason the 6.0 grams of calcium carbonate per 1000 grams of the standard McCollum diet were omitted leaving 2.56 grams of calcium per 1000 grams of food contained in other constituents of the formula. This amount of calcium is still appreciable but could not be reduced any further without a complete change to a diet inadequate in other respects.

For several weeks before parathyroidectomy and for at least 40 days afterwards, daily records were taken of the intake of water and the calcium lactate solution.

RESULTS

Effect of parathyroidectomy on calcium intake. Parathyroidectomy produced a definite increase in the intake of calcium lactate solution and a decrease in the water intake. In the typical record presented in figure 1 the calcium lactate intake increased from a level of 4.8 cc. per day before parathyroidectomy to 27.6 cc. 40 days later; the water intake, on the contrary, decreased from 28.2 to 8.5 cc.

The daily average of calcium lactate intake for the 18 rats was 3.9 times as high after parathyroidectomy as before. See table 1. In 1 animal it was 12.9 times as high (21.9 cc. as compared with 1.7 cc.). The average daily

water intake decreased from 19.5 cc. for the 10-day period before parathyroidectomy to 14.7 cc. for the 30- to 40-day period afterwards, a decrease of 25 cent per undoubtedly due to the water ingested in the calcium lactate solution.

The animals were all normal so far as we were able to determine, showing normal growth curves and no signs of tetany.

Effect of parathyroid implants on increased calcium appetite. It occurred to us that the dependence of the increased calcium appetite on the removal of the parathyroid glands would be definitely established if replacement therapy with parathyroid implants caused the appetite to return to a normal level again. Accordingly, 6 parathyroidectomized rats received 2 parathyroid implants

TABLE 1. CHOICE OF 2.4% CALCIUM LACTATE SOLUTION OR WATER.
(Dry McCollum Diet without Calcium Carbonate)

Rat	Age in days at parathyroidectomy	Average daily tap water intake in cc.		Average daily calcium lactate intake in cc.		Ratio of calcium intake 30–40 days after: 10 days before
		10 days before parathyroidectomy	30–40 days after parathyroidectomy	10 days before parathyroidectomy	30–40 days after parathyroidectomy	
1	84	22.5	5.0	2.5	19.0	7.6
2	84	26.0	20.0	6.4	11.8	1.8
3	84	25.5	15.0	3.4	19.3	5.6
4	84	29.3	32.2	2.9	10.2	3.5
5	85	21.8	18.2	5.3	19.0	3.6
6	84	24.6	23.2	5.0	20.0	4.0
7	43	11.2	10.9	5.4	14.1	2.6
8	44	13.0	11.9	5.4	19.6	3.6
9	44	12.1	14.8	3.7	17.3	4.7
10	45	9.1	7.5	8.1	29.1	3.6
11	44	8.5	7.6	12.4	23.2	1.9
12	45	10.3	16.8	9.1	23.7	2.6
13	82	20.6	10.3	3.4	11.9	3.5
14	82	16.9	10.2	12.1	20.2	1.7
15	82	33.4	17.6	1.7	21.9	12.9
16	82	21.1	17.9	12.3	12.4	0.1
17	78	17.2	16.2	10.9	20.0	1.8
18	78	28.2	8.5	4.8	27.6	5.8
Average	70	19.5	14.7	6.4	18.9	3.9

(donors 8 and 34 days of age) into the anterior chamber of each eye, after the technique described for uterine implants by Markee (3).

Five of these animals showed an immediate decrease in intake of calcium lactate solution and an increase in water intake. In the record in figure 2 it will be seen that the intake of calcium lactate which had increased from 8.1 cc. before operation to 29.1 cc. 40 days later, decreased within 15 days after implantation of parathyroid glands practically to its original level; water intake, which had decreased from 9.1 cc. before operation to 7.5 cc. 40 days later, reached a peak of 45.0 cc. 14 days after implantation.

In 2 animals the calcium appetite was still very low 37 days after the implants were made. The implants removed from these animals at autopsy showed normal parathyroid tissue. Two implants with normal parathyroid cells

were contained in this graft, 1 entirely and 1 partly surrounded by thyroid tissue regenerated from unintentionally implanted thyroid fragments. In the other 3 animals the calcium appetite was low for approximately 15 days before

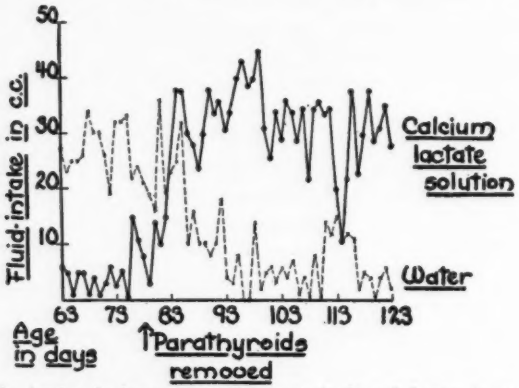


Fig. 1. Graphs showing the increase in calcium lactate intake and decrease in water intake produced by parathyroidectomy.

it returned to a high level again. The implants removed from these animals contained no parathyroid tissue. No implant was found in the animal which showed no change in calcium appetite.

Elimination of increased calcium need through replacement therapy with parathyroid implants, then, caused the calcium appetite to decrease to a normal level.

Cyclical fluctuations in calcium appetite. It is worthy of note that a fairly

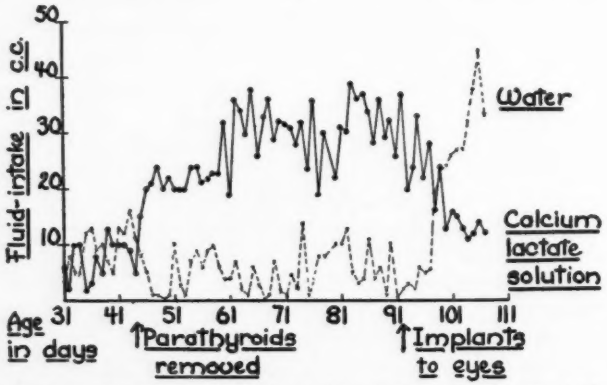


Fig. 2. Graph showing the effect produced on calcium lactate intake and water intake by parathyroidectomy and subsequent implantation of parathyroid glands.

regular cycle of calcium appetite was observed in 2 animals after parathyroidectomy. A record of 1 of these animals presented in figure 3 (top), shows a cycle with a frequency of 15 to 20 days. It is of interest (bottom chart) that

these cycles were present and even more marked in the spontaneous activity, as measured by the revolving drum method. We are unable at present to offer any interpretation of this phenomenon. Similar cyclical fluctuations were observed in a previous study of the effects produced on activity by removal of the thyroid and the parathyroid glands (4). However, these cycles, were of longer duration with a frequency of 25 to 35 days.

Calcium needs of normal animals. The parathyroidectomized rat increased its calcium intake according to its calcium need. How closely will the normal rat adjust its calcium intake to the amount found, empirically, by McCollum to be necessary for normal growth and development?

The standard McCollum diet contains 8.56 grams of calcium per 1000 grams of food, which means that with the average daily food-intake of 14.5

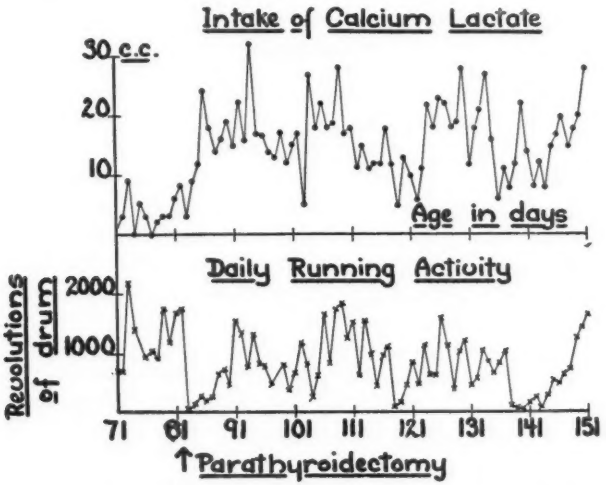


Fig. 3. Graphs showing cycles of calcium lactate intake and running activity.

grams, the calcium intake per day is 0.124 grams. In the present experiments the average daily intake of calcium lactate solution (see table 1) was 6.4 cc. representing a daily calcium intake of 0.020 gram per day and the maximum reached by 1 animal was 12.4 cc., representing 0.039 gram of calcium per day. In addition every animal received an average of 0.036 gram of calcium per day in the dry diet. The total intake then was 0.056 gram, and the maximum 0.075 gram which is still only slightly more than half the average amount received in the McCollum diet. The average daily calcium intake of the parathyroidectomized rats, 30 to 40 days after operation, 0.091 grams, was lower than the amount calculated by McCollum to be necessary for normal animals. However, these discrepancies are not great; it is of interest that the amount of calcium taken spontaneously by the animals agrees even this closely with the amount arrived at empirically by McCollum.

REMARKS

It is possible that parathyroidectomized rats will take an even greater amount of calcium offered in some other solution than calcium lactate. The possibility must also be considered that parathyroidectomized rats may have needs in addition to calcium; that they may show an increased appetite for other minerals, or even for the different elements of the diet, the proteins, fats and carbohydrates.

Furthermore, they may show an avèrsion for certain substances rather than a predilection. Shelling (5) has shown that parathyroidectomized rats freely ate diets rich in calcium but refused diets rich in phosphorus. We have found in experiments in which a free choice was offered of calcium lactate and sodium phosphate solutions that after parathyroidectomy an increase in calcium lactate intake was associated with a definite decrease in sodium phosphate intake.

DISCUSSION

Furher evidence has been obtained in these experiments that deficiencies and needs produced by glandular removal are definitely reflected in changes in appetite; in other words, that appetite actually guides the animals to ingest substances which help to correct the deficiencies as well as to maintain normal growth and health. By means of this technique a definite measure of the appetite for specific substances may be obtained, thus making possible a quantitative study of the needs produced by glandular disturbances.

It is obvious that selection of different substances by animals, ordinarily controlled by appetite, must ultimately be dependent on the taste mechanism. By means of this technique an objective study may be made of the sense of taste, not only of the ability of the animal to differentiate between different tastes, but through surgical interference, to determine the central and peripheral nervous components upon which taste depends.

SUMMARY

Appetite for calcium was definitely increased by parathyroidectomy in 17 of 18 rats. The average daily intake of a 2.4 per cent solution of calcium lactate for the animals was 3.9 times as high after parathyroidectomy as before. Replacement therapy with parthyroid implants in 5 animals to the anterior chamber of the eye caused the calcium appetite to return to normal.

The calcium needs of 18 normal rats determined by the appetite method averaged 0.056 gram per day with a maximum of 0.075 gram which is somewhat lower than the amount, 0.124 gram, calculated empirically by MacCallum to be necessary for normal growth. Further evidence is thus obtained showing that appetite is significant in studies of deficiencies created by glandular disturbances or extirpation.

REFERENCES

1. Richter, C. P.: Am. J. Physiol. **115:** 155. 1936.
2. MacCallum, W. G., and C. Voegtlin: J. Exper. Med. **11:** 118. 1909.
3. Markee, J. E.: Am. J. Physiol. **100:** 374. 1932.
4. Richter, C. P.: Endocrinology **17:** 73. 1933.
5. Shelling, D. H.: J. Biol. Chem. **96:** 195. 1932.

FURTHER STUDIES ON THE ACTION OF PARATHYROID EXTRACT IN THE DOG FOLLOWING TOTAL AND PARTIAL ABLATION OF THE KIDNEYS

W. R. TWEEDY, R. D. TEMPLETON AND F. A. McJUNKIN
From the Departments of Physiological Chemistry, Physiology, and Pathology, Loyola University School of Medicine
CHICAGO, ILLINOIS

The complete elucidation of the clinical entity known as renal rickets is dependent upon a better understanding of the functional relationship of the kidneys and the parathyroids. In this connection the importance of studies on the action of parathyroid extract in the nephrectomized animal is apparent.

In a recent publication Ellsworth and Futcher (1) reported that the serum calcium of the bilaterally nephrectomized dog can be increased by the injection of parathyroid hormone. However, Tweedy, Templeton, and McJunkin (2), who carried out a long series of experiments, were unable to obtain conclusive evidence of the action of exogenous parathyroid hormone in increasing the serum calcium of the nephrectomized dog. Further evidence is presented in this paper that the characteristic action of exogenous parathyroid hormone is not manifested by an increase in serum calcium in the absence of kidney tissue.

EXPERIMENTAL

Bilateral nephrectomy followed by injection of calcium gluconate and parathyroid extract. In our first publication (2) methods were discussed and a control series of 5 animals was described. It was found that 4 of the 5 control animals showed an increase in serum calcium of 1.75 to 2.32 mg. per cent within 24 hours after nephrectomy. Our failure to demonstrate any appreciable increase in serum calcium after the injection of parathyroid extract suggested the possibility that in the completely nephrectomized animal there had occurred an impairment of the mechanism, or mechanisms, by which calcium salts are made available to the blood. The question appeared to be whether calcium salts injected, intramuscularly, into the bilaterally nephrectomized dog would be taken up by the blood and maintained in higher concentration for a longer time if exogenous parathyroid hormone was also made available by injection.

Accordingly, 4 dogs (table 1) were bilaterally nephrectomized. Each was injected, intramuscularly, with 20 cc. of 10 per cent calcium gluconate immediately after the operation. Two of the animals were also injected, subcutaneously, with parathyroid extract.

The authors are indebted to Eli Lilly and Company for the parathyroid extract used in these experiments, and to Messrs. C. Vicens-Rios, G. H. Smullen, and L. Herdegen for their assistance in the operations and autopsies.

Bilateral ligation of the ureters followed by the injection of parathyroid extract. In view of the apparent negative effect of parathyroid extract after bilateral nephrectomy, we varied the operative procedure for the elimination of kidney function. Some degree of kidney action was still possible and, as will be seen, the results obtained were irregular and slightly suggestive of hormone action. A description of the expriments follows.

Dog 5 (table 2) showed an increase in serum calcium of only 0.65 mg. per cent 16 hours after the injection of 100 U. of parathyroid extract, thus indicating the ineffectiveness of the parathyroid extract in increasing the serum calcium while the animal's ureters were ligated. The animal's ureters, which had been brought to the outside of the abdominal wall, were cut in order to

TABLE 1. SERUM Ca AND INORGANIC P VALUES IN BILATERALLY NEPHRECTOMIZED ANIMALS IN WHICH CALCIUM GLUCONATE WAS INJECTED (DOGS 1–4), AND IN WHICH PARATHYROID EXTRACT WAS INJECTED (3–4).

Time	Dog 1, wt. 15 kg.		Dog 2, wt. 17 kg.		Dog 3, wt. 14.5 kg.			Dog 4, wt. 15 kg.		
	Ca	Inorg. P	Ca	Inorg. P	Hormone Dose	Ca	Inorg. P	Hormone Dose	Ca	Inorg. P
hr.	mg.%	mg.%	mg.%	mg.%	U.	mg.%	mg.%	U.	mg.%	mg.%
−1	11.21	7.59	12.18	7.43		10.87	5.45		10.97	
¼					100			100		
1	11.97	5.70							10.24	8.20
2	13.79	8.56	12.43	10.90						
3	13.35	7.50	15.10	7.14					14.32	6.00
5			15.43	6.76					14.32	10.15
6	12.56	8.56								
9									15.41	9.82
10	12.38	8.56								
11			14.85	8.00		13.18	8.68			
24			13.11	9.08		12.61	6.66	50	14.90	21.00
25	14.56	10.90								
33	15.73	11.64						50	15.39	16.00
49	15.58	15.00	11.89	21.42		14.34	10.42		17.39	
55	15.63	25.40	13.20	21.04				75	16.50	22.84
70						14.74	12.00		17.08	17.00
76									17.96	29.24

allow the escape of any urine which had formed or might form during the next experimental period. There was a marked flow of urine, indicating the accumulation of considerable urine in the ligated ureters and kidney pelvi. Immediately afterward 100 U.[1] of parathyroid extract were injected, subcutaneously. As shown in table 2, the serum calcium increased from 10.43 mg. per cent to 15.49 mg. per cent during the ensuing 40 hours, while the serum inorganic phosphate increased from 12.32 to 15.49 mg. per cent, thus indicating the restoration of conditions which were favorable for hormone action.

Dog 6 (table 2) showed an increase in serum calcium of 3.86 mg. per cent, 16 hours after ligation of the ureters and the injection of 68 U. of parathyroid extract. Eight hours later the ureters were cut and a small amount of urine flowed out. Sixteen hours later the calcium level had dropped until it was only 0.69 mg. per cent above the initial value. Forty hours later the

[1] Units as defined by J. B. Collip and E. P. Clark, J. Biol. Chem. 64: 485. 1925. One of these units is equivalent to 5 Hanson units.

animal was found dying. The increase in serum calcium in this animal exceeded the maximum increase in serum calcium observed in the animals, which were subjected to nephrectomy alone and it seems quite possible that the hormone injected may have been partly effective.

In order to obviate as much as possible the toxic effects produced by ligation of the ureters, we attempted to bring about a rapid mobilization of calcium salts into the blood. Animal 7 (table 2) was injected, intravenously, with 100 U. of parathyroid extract immediately after ligation of the ureters. Approximately 4 hours later 100 U. more were injected, intravenously. During the first 11 hours the serum calcium increased only 1.06 mg. per cent, and at the end of 24 hours was only slightly higher. The amount of hormone injected should have produced an increase of at least 5 mg. per cent in the serum calcium of the average normal dog of the same weight.

TABLE 2. INJECTION OF PARATHYROID EXTRACT AFTER BILATERAL LIGATION OF THE URETERS SERUM Ca AND INORGANIC P VALUES.

Time	Dog 5, wt. 18 kg.			Dog 6, wt. 13.6 kg.			Dog 7, wt. 14.5 kg.		
	Hormone Dose	Ca	Inorg. P	Hormone Dose	Ca	Inorg. P	Hormone Dose	Ca	Inorg. P
hr.	U.	mg. %	mg.%	U.	mg. %	mg. %	U.	mg. %	mg. %
−1		10.43	6.00		12.35	7.50		12.47	4.36
¼	100*			68			100(i)		
							100(i)	12.75	5.44
6								13.53	6.83
11								13.38	7.28
16		11.08	10.42		16.21	12.24			
24	100	10.43	12.32					13.53	8.00
40		12.08	15.36		13.04	12.00			
64		15.49	13.32						

* Administered subcutaneously unless designated (i), intravenously.

Unilateral nephrectomy followed by the injection of parathyroid extract. The temporary disturbance produced in the normal metabolic processes of the dog by unilateral nephrectomy does not inactivate the mechanism, or mechanisms, which normally may be affected by the injection of parathyroid extract. This is shown in the following experiments.

The serum calcium and the serum inorganic phosphate of a dog were 10.04 and 6.00 mg. per cent, respectively. Four hours after unilateral nephrectomy these values were 10.14 and 5.45 mg. per cent, respectively. At that time 100 U. of parathyroid extract were injected, subcutaneously. During the ensuing 17 hours the serum calcium of this animal (dog 8) increased to 12.96 mg. per cent while the inorganic phosphate decreased to 3.38 mg. per cent. Within the next 24 hours the serum calcium level reached 15.62 mg. per cent, while the serum inorganic phosphate increased to 8 mg. per cent. Sixty-five hours after the injection of parathyroid extract the serum calcium had decreased to 11.67 mg. per cent, while the serum inorganic phosphate had decreased to 6.66 mg. per cent. The blood picture presented with respect to the rise and fall of serum calcium and serum inorganic phosphate is typical of parathyroid hormone action.

The reaction of the second dog to parathyroid extract was tested on 3 separate occasions. Four days previous to unilateral nephrectomy, the reaction of this animal (dog 9) to a dose of parathyroid extract was manifested by an increase of 4 mg. per cent in its serum calcium. At the time of operation the serum calcium was 13.18 mg. per cent, while the serum inorganic phosphate was 6.00 mg. per cent. Five hours after unilateral nephrectomy the serum calcium was unchanged, but the serum inorganic phosphate had increased to 8.56 mg. per cent. At that time 60 U. of parathyroid extract were injected, subcutaneously. During the ensuing 17 hours the serum calcium and the serum inorganic phosphate of this animal changed to 20.85 and 7.66 mg. per cent, respectively.

OBSERVATIONS AT NECROPSY

Bilaterally nephrectomized dogs, which had been injected with calcium gluconate (1-4), and with parathyroid extract (3-4). From gross observations all of the organs of dog 1 were normal in appearance. The gall bladder of dog 2 was greatly distended; otherwise there was no suggestion of pathology. There were no abnormal gross changes in the organs of dog 3, except the heart which was flabby in consistency. In dog 4, small hemorrhagic patches were seen in the mucosa of the stomach.

By microscopic examinations the stomach, lungs, and heart of dogs 1, 2, and 3 were normal in appearance. There was slight fatty degeneration of the liver of dog 4. The pancreas and lungs were normal. In the stomach there was necrosis and calcification of individual cells of the glandular epithelium. There was congestion but no hemorrhage was present in the section examined.

Bilateral ligation of the ureters in dogs injected with parathyroid extract. Microscopically, in the kidney of dog 5 there was tubular necrosis with calcification. The average lesion involved 3 parallel tubules and showed necrosis with here and there heavy deposition of calcium. The lesions were identical with those which we have found in the rat kidney after parathyroid hormone overdosage (3). The appearance of glomerular distension of capsular space seen in early hydronephrosis was not present in the cortex, either inside or outside of the necrotic areas.

Sections from the kidneys and spleen of dog 6 were examined microscopically. The kidneys showed nephrosclerosis of the arteriolar type. The spleen was normal. No observations were made on the tissues of dog 7.

Unilateral nephrectomy in dogs injected with parathyroid extract. No observations were made on the tissues of dog 8. Microscopic observations on the tissues of dog 9. In the kidney there were large areas of necrosis with calcium deposits here and there. The heart showed suppurative myocarditis with numerous small abscesses.

SUMMARY

In a previous investigation (2) in which bilaterally nephrectomized dogs were injected with parathyroid extract we failed to find conclusive evidence of its action in either the blood or other tissues. The experiments presented here (table 1) confirm our first observations, and also indicate that the dis-

tribution of injected calcium salts between the blood and other tissues is unaffected by the injection of parathyroid extract. The microscopic findings also indicate the absence of hormone effect. Only in dog 4 was there the slightest suggestion of a hormone lesion. In this animal the scattered individual necrotic cells of the stomach mucosa presented a minor change that fell far short of the usual destructive hemorrhagic hormone lesions, which would have developed in the stomach of the intact dog under comparable conditions. These pathological findings confirm our previous observations that destructive lesions of the soft tissues are essentially absent in dogs devoid of all kidney action, regardless of the amount of parathyroid extract injected.

The relative non reactivity of dogs 5 and 7, as judged by serum calcium analyses, appears to be directly traceable to the rapid development of renal insufficiency after ligation of the ureters. This is particularly evident in dog 5, which showed a rapid rise in serum calcium after the ureters were reopened, and another injection of parathyroid extract had been made.

The data show that the unilaterally nephrectomized dog reacts to an injection of parathyroid extract in a manner comparable to the normal dog.

REFERENCES

1. Ellsworth, R., and P. H. Futcher: Bull. Johns Hopkins Hosp. **57**: 91. 1935.
2. Tweedy, W. R., R. D. Templeton and F. A. McJunkin: Am. J. Physiol. **115**: 514. 1936.
3. McJunkin, F. A., W. R. Tweedy and H. C. Breuhaus: Arch. Path. **14**: 649. 1932.

EXCRETION OF MALE HORMONES I.[1]

CHARLES D. KOCHAKIAN[2]
Department of Vital Economics, University of Rochester
ROCHESTER, NEW YORK

Funk, Harrow and Lejwa (1) found that men in the prime of life excreted in the urine 4 to 5 times as much male hormone as men from 50 to 70 years of age. Buhler (2) found a similar relationship between age and quantity. Womack and Koch (3) reported that the urine of boys under 10 years of age contained no male hormone, urine of adolescent boys contained the same amount as that of adult men, and that normal and pregnant women excreted as much male hormone as mature men. This latter finding has been abundantly confirmed (4, 5, 6, 7). On the other hand it has been demonstrated that the male organism excretes large amounts of female sex hormone (8). In fact the stallion (9) excretes more female sex hormone than any other organism and the testis of the stallion is the richest known tissue source of this hormone. Zondek (10) believes that the stallion metabolises the male hormone as soon as formed to female hormone. However, Zondek (11) also believes that the female hormone has an extragonadal but endogenous as well as a gonadal origin. This has been amply supported by Bingel (12) and Eng (13) who found that castrated men and women excrete large amounts of female hormone which is endogenous in origin. Bingel (12) also found that castrated men excrete small quantities of male hormone.

PROCEDURE

In this paper are reported the assays of *A)*, the composite urine of young medical students; *B)*, the urine of individual medical students; *C)*, the urine of old men; *D)*, the urine of a castrated man, a normal man, rabbits and castrated dogs after injection of male hormone.

The urine extracts were all prepared according to the method of Kochakian and Murlin (14) except as noted otherwise in the text. The extracts were dissolved in oil and assayed on capons. One capon[3] unit (C.U.) is the amount of hormone which, when injected daily on 2 successive days, will produce on the 3rd or 4th day an average maximum increase of 3 to 4 mm. in length plus height (L+H) of the combs of at least 3 white leghorn capons. Invariably more than 3 capons were used for each assay.

---

[1] This investigation was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] This report is taken from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Rochester, June 1936.

[3] Henceforth we shall use the term capon unit instead of bird unit which we used in previous publications (Proc. Soc. Exper. Biol. & Med. 32: 1064. 1935: J. Nutrition 10: 437. 1935. This change is proposed because we feel that capon unit is more descriptive and specific than bird unit.

In the latter part of this investigation a more sensitive method of assay was found to be desirable, hence it was supplemented by the Fussganger smear technic (15). Whenever possible both methods were employed simultaneously. All results are expressed according to the injection technic.

Male Hormone Content of Composite Urine of Medical Students. The urine which was collected continuously from December 1933 to June 1934, was extracted in batches varying from 200 to 982 liters each. The extracts were dissolved in olive oil so that 1 cc. of the solution was equivalent to 4 liters of urine.

The results for the 9 different preparations gave an average value of 6-8 C.U. per liter, regardless of time of year, hence no seasonal factor is apparent.

Male Hormone Excretion by Young Men. The constancy of the male

TABLE 1. MALE HORMONE EXCRETION BY INDIVIDUAL MEDICAL STUDENTS.

Subject Age yr.	mm. L+H per 24 hr.	Per cent Deviation from Average	C.U. per 24 hr.
21	38	−20	10–13
22	24	−50	6– 8
23	49	+ 2	12–16
24	108	+125	27–36
24	49	+ 2	12–16
24	39	−19	10–13
24	58	+20	14–19
25	36	−25	9–12
26	57	+19	14–19
27	40	−17	10–13
27	44	− 8	11–14
29	33	−30	8–11
Averages	48	−20 to +11	12–16

hormone content of the composite urine of medical students prompted the investigation of the male hormone content of the urine of individual medical students. The samples were collected quantitatively for successive 24-hour periods under carefully controlled conditions.[4] Halves of pooled 3-day collections were extracted. The extracts were all dissolved in the same amount of olive oil for assay.

The results (table 1) show an average daily excretion of 12 to 16 C.U. per day. The average deviation for the group, excluding the two extremes (subjects 2 and 4) is —20 to +11. There is no relationship apparent between height or weight. The other factor, age, seems to be the controlling element.

Male Hormone Excretion by Old Men. In order to obtain a relationship of the excretion of male hormone by old men and young men, the urine of 5 men, aged 50 to 76 years, was obtained. All of these men had hypertrophied prostates[5] of varying degree, except subject 5, who had a normal prostate. It is not, however, the purpose of this report to draw a comparison between the

[4] I am indebted to Professor H. B. Pierce for arrangements for the collection of urine samples.
[5] I am indebted to Drs. C. F. Schroeder and D. Jarman of the Strong Memorial Hospital resident staff for these urine samples.

amount of male hormone excreted by old men with normal prostates and men with hypertrophied prostates. The relationship of male hormone to prostatic hypertrophy constitutes a problem in itself and is under active investigation here.

The results (table 2) indicate that these men excrete a small amount of hormone. The average excretion was only 2 to 3 C.U. per day, or 1/6 that excreted by young men.

The Excretion of Injected Male Hormone. In this phase of the problem two sources of male hormone for the injections were used, *1)*, urinary extracts which are reported in the first part of this paper, and *2)*, the synthetic male hormone, androstendion, prepared according to the methods proposed independently by Ruzicka and Wettstein (6) and Butenandt and Kudszus (17).

In the experiments in which either a single injection or 3 successive injections were administered, 2 or 3 urine samples were obtained. One sample was obtained from the time of injection to at least 72 hours after injection and was

TABLE 2. MALE HORMONE EXCRETION BY OLD MEN.

Age yr.	Description	Av. L+H in mm./24 hr.	C.U./24 hr.
50	Hypertrophied prostate	3	0.8–1
55	Hypertrophied prostate	6	1.5–2
60	Hypertrophied prostate	8	2–3
62	Normal prostate	12	3.0–4
76	Hypertrophied prostate	12	3.0–4
	Averages	8	2–3

followed by one or two equivalent periods. When injections were administered over a period of time, the urine samples were collected for the whole or part of the duration of the injections.

The urine samples were extracted in the usual manner except in experiments 9, 10 and 11. In these a modification was made in the alkali treatment of the chloroform residue in order not to destroy any androstendion that might be excreted as such. Androstendion, because of its α, β, unsaturated ketone grouping, is labile to alkali. The chloroform residue was dissolved in ether and extracted 3 times with 5 per cent NaOH solution instead of heating with 20 per cent NaOH solution and then extracting the diluted alkaline mixture with ether.

The human subjects were patients in Strong Memorial Hospital and the animals were kept in metabolism cages. The results are compiled in table 3.

Experiments 1 and 2 were on the same subject, a 50 year-old male who was in the hospital with a case of hypertrophied prostate. He received 2 injections of male hormone; the amount of male hormone excreted, however, in both instances was no greater than in the periods in which he had not received any hormone.

Experiment 3 was on a human castrate who was maintained on division in Strong Memorial Hospital. A urine sample was collected for 8 days before

injection and was assayed on 2 standardized capons and one slip[6] which was known to react with at least twice the response of the standard birds. Administration of a daily dose equivalent to 27 hours of urine to each of the capons for 2 successive days did not produce the slightest response even in the slip.

TABLE 3. THE URINARY EXCRETION OF INJECTED MALE HORMONES.

No. Expt.	Subject	Injections		Total C.U. Injected	Date of urine sample	C.U. Excreted	Per cent Recovered
		Date	C.U./day				
1*	50 yr. old male	—			2/23–3/1/35	±1	0
		3/1/35	11–17	11–17	3/2–3/6/33	±1	0
		—			3/6–3/9/35	±1	
2*	50 yr. old male	3/9/35	11–17	11–17	3/9–3/12	±1	0
		—			3/12–3/14	±1	
3*	50 yr. old male castrate	—			10/9–10/17	0	
		10/17–10/23	24–32				
		10/24–11/2	30–40	613–819	10/17–11/8	18–23	3
		11/3–11/7	29–39				
4*	Dog 3 castrate	4/24/35	125–170	125–170	4/24/–4/28	6–8	5
					4/28–4/31	0	0
5*	Dog 2 castrate	4/26/35	208–310	208–310	4/25–4/29	5–7	2
					4/29–5/3	0	0
					5/3–5/7	0	0
6*	Dog 3 castrate	5/9/35	235–319	235–319	5/8–5/11	4–5	2
					5/11–5/14	0	0
					5/14–5/17	0	0
7*	Rabbit 1001	1/16–2/6	2.4–3.2	50–67	1/16–2/6/36	3–4	6
8*	Rabbit 1002	1/14–1/31	4.8–6.4	108–143	1/14–1/31	3–4	3
	1003	2/8–2/9	2.4–3.2		2/8–2/19		
9†	Dog 2 castrate	2/17	32–42		2/17–2/24	4–6	4
		2/18	32–42	96–126	2/24–2/30	±1	±1
		2/19	32–42				
10†	Dog 2 castrate	3/12	96–126		3/12–3/19	5–7	2
		3/13	96–126	288–378	3/19–3/29	±1	±0.4
		3/14	96–126				
11†	Dog 1 castrate	4/6	96–126		4/5–4/13	7–9	2
		4/7	96–126	288–378	4/13–4/25	±1	±0.4
		4/8	96–126		4/25–5/4	±0.5	±0.1
12	Dog 2 castrate		none	none	5/30–6/6	±0.3	

* Male hormone urinary extracts.
† Androstendion.

Apparently no male hormone was being excreted. The administration of 613 to 819 C.U. of urinary male hormone extract from October 17 to November 7, 1935, gave in the urine a total of 18 to 23 C.U. or only 3 per cent of the amount injected; 97 per cent of the hormone remained unaccounted for.

Experiments 4, 5 and 6 on castrated dogs showed a similar loss of the injected hormone, also that subsequent periods showed no activity. From use

[6] A slip is an incompletely castrated rooster.

of the capon comb-growth injection method it appeared, therefore, that the small amount of male hormone appearing in the urine was excreted within the first 72 hours after injection and that castrate dogs do not spontaneously excrete male hormone.

Experiments 7 and 8 were on rabbits which had a Brown-Pierce tumor of the testis, but the results obtained are again in close agreement with the results obtained on the human subjects and the dogs.

Experiments 9, 10 and 11 were further experiments on castrate dogs with androstendion as the male hormone. The results in these experiments are in perfect agreement with those obtained from the use of the urinary hormone

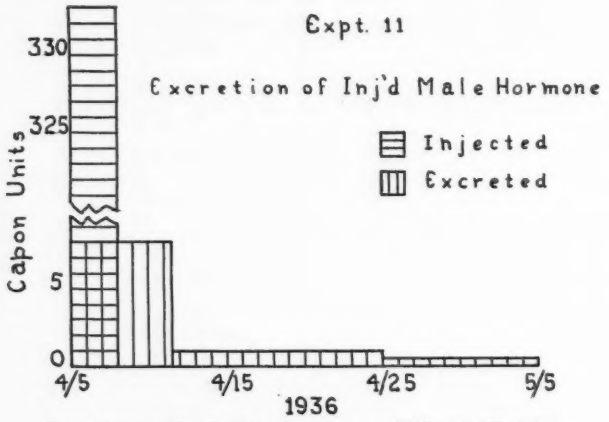


FIG. 1. Average values for the capon units are used (cf. table 3 Expt. 11).

extracts. The use, however, of the more sensitive Fussganger smear technic of assay for the 2nd and 3rd samples of these experiments, revealed that active material, though slight, was being excreted as much as 17 days (experiment 11) after the last injection (fig. 1). To make certain that the excretion of this small amount of male hormone was not due to excretion of stored injected hormone, dog 2, on May 28, 44 days after receiving the last male hormone injection, was placed on a diet of Purina dog chow. From May 30 to June 6 the urine was collected and assayed for male hormone by the smear technic. The amount was 0.3 to 0.4 C.U. for the 7 days or an average value of 0.05 C.U. per day, which is similar to the daily output of male hormone obtained in the third urine sample of dog 1 in experiment 11. It seems, therefore, that castrated dogs do excrete small amounts of male hormone. The inability to detect this before was due to the lack of a sensitive method of assay.

DISCUSSION

The findings of previous workers that the urinary excretion of male hormone by males decreases with age has been confirmed. The study of the excretion of male hormone by young men furnishes a standard which is necessary if a proper interpretation of clinical findings is to be made. Such a stand-

ard, however, must not be accepted too dogmatically until it has been confirmed and extended by other laboratories.

The detection of male hormone in the urine of castrated animals, by the smear technic of assay, not only indicates an extragonadal source of male hormone, but also emphasizes the importance of sensitive methods of assay; for male hormone could not be detected under similar circumstances when the much less sensitive injection technic of assay was used. The extragonadal source of male hormone substance is now under investigation.

In no instance was there any appreciable amount of male hormone recovered from the urine of animals or men subcutaneously injected with oil solutions of the substance. The amount excreted was only 2 to 6 per cent of the amount injected, regardless of the chemical nature of the male hormone used. Preliminary investigations of the feces indicate that an approximately equivalent amount is excreted by the alimentary tract. The greater part, approximately 90 per cent of the injected material, therefore, remains to be accounted for.

To speculate on the fate of the hormone lost after injection, is as yet premature. It seems quite possible that the body metabolises the hormones to some inactive or less active forms and the small amount excreted after the injections is a waste product or products of this mechanism. This hypothesis is supported by the fact that the substances excreted in the urine, androsterone (18), dehydroandrosterone (19) and probably isoandrosterone (20) are not only chemically different from the substance produced in the testes, testosterone, but also physiologically less active (21). The chief chemical difference between the active materials from the two natural sources is the lability of testosterone to alkali (22), which is due to the $\alpha\ \beta$ unsaturated ketone group possessed by testosterone. This difference, therefore, provides a means of determining whether injected testosterone is metabolised in the body and excreted as an alkali-stable substance. In view of the fact that the male organism at all times excretes estrogens, it is also probable that some of the lost hormone is inactivated by conversion to this form.

The first of these possibilities is now under investigation with the purpose of isolating and identifying the substance or substances excreted. It is hoped also, to investigate the question of conversion to estrogens.

SUMMARY

The composite urine of a group of young men between the ages of 22 and 29 years was found to contain 6 to 8 C.U. of male hormone per liter. This amount was found to be constant from December 1933 to June 1934.

Twelve medical students, 22 to 29 years old, excreted an average of 12 to 16 C.U. of male hormone per day with an average deviation of —20 per cent to +11 per cent. Two of the 12, however, showed a deviation of +125 per cent and —50 per cent.

Five old men 50 to 76 years of age excreted an average of 2 to 3 C.U. of male hormone per day or 1/6 the amount excreted by young men.

Male hormone could not be detected in the urine of a castrate man and 2

castrate dogs by the injection technic of assay. It was, however, detected in small amounts in the urine of castrate dogs by the smear technic of assay.

Urinary male hormone extracts and synthetic androstendion injected subcutaneously into men, rabbits and dogs, could be recovered to the extent of only 2 to 6 per cent in the urine. It is suggested that the lost hormone is converted to less active forms and also possibly estrogens.

It is a pleasure to acknowledge here the constructive criticism of this paper by Professor John R. Murlin.

REFERENCES

1. Funk, C., B. Harrow and A. Lejwa: Proc. Soc. Exper. Biol. & Med. **26:** 569. 1932.
2. Buhler, F.: Ztschr. f. d. ges. exper. Med. **86:** 650. 1933.
3. Womack, E. B., and F. C. Koch: Endocrinology **16:** 273. 1932.
4. Loewe, S., H. E. Voss and E. Rothschild: Biochem. Ztschr. **237:** 214. 1931.
5. Kabak, J. M.: Endokrinologie **9:** 84. 1931.
6. Kabak, J. M.: Endokrinologie **10:** 12. 1932.
7. Siebke: Arch. f. Gynäk. **56:** 317. 1934.
8. Fee, A., G. F. Marrian and A. S. Parkes: J. Physiol. **67:** 377. 1929.
9. Zondek, B.: Nature **133:** 209. 1934.
10. Zondek, B.: Nature **133:** 494. 1934.
11. Zondek, B., and H. v. Euler: Skandinav. Arch. f. Physiol. **67:** 259. 1934.
12. Bingel, A.: Klin. Wchnschr. **14:** 1827. 1935.
13. Eng, H.: Klin. Wchnschr. **15:** 349. 1936.
14. Kochakian, C. D., and J. R. Murlin: J. Nutrition **10:** 437. 1935.
15. Fussgänger, R.: Med. und Chem. (I. G. Farbenindustrie) Leverkusen, 194. 1934.
16. Ruzicka, L., and A. Wettstein: Helv. Chim. Acta **18:** 986. 1935.
17. Butenandt, A., and H. Kudszuz: Ztschr. f. physiol. Chem. **237:** 75. 1935.
18. Butenandt, A., and K. Tscherning: Ztschr. f. physiol. Chem. **229:** 167. 1934.
19. Butenandt, A., and H. Dannenbaum: Ztschr. f. physiol. Chem. **229:** 192. 1934.
20. Butenandt, A., and H. Cobler: Ztschr. f. physiol. Chem. **234:** 218. 1935.
21. David, K., E. Dingemanse, J. Freud and E. Laqueur: Ztschr. f. physiol. Chem. **233:** 281. 1935.
22. Gallagher, T. F., and F. C. Koch: J. Biol. Chem. **104:** 611. 1934.

EFFECTS ON SPERMATOGENESIS OF A FOLLICLE-STIMULATING EXTRACT OBTAINED FROM MENOPAUSAL OR CASTRATE URINES[1]

JOHN HUBERMAN, HOWARD H. ISRAELOFF AND BENJAMIN HYMOWITZ

From the Department of Endocrinology, Newark Beth Israel Hospital
NEWARK, NEW JERSEY

The discovery of a gonad-stimulating or gonadotropic hormone in human urine in certain cases of menopause and castrate women has further complicated the endocrine picture of the reproductive process. This principle appears to be gonadotropic and, in laboratory animals, has stimulated germ cell division. The most striking results with castrate and menopausal urine extracts have been obtained in the male monkey and consisted of stimulation of spermatogenesis (1, 2, 3). With this in mind, we investigated the effects of these extracts on spermatogenesis in human adults. So far as we know, no survey of the influence of this extract on humans has been reported.

This castrate and menopausal urine extract can be described as a polypeptid-like substance, which apparently is either identical with, or very similar to the gonadotropic hormone from the anterior lobe itself. It is prepared from the urine of certain castrates and post-menopausal women and is standardized by the production of large numbers of follicles in the ovaries of 30-day old rats, 100 hours after 6 injections, distributed over a 3-day interval. The units do not correspond to those obtained for pregnancy urine, using the same type of animals. This extract is prepared under a trade name.[2]

We selected cases of male sterility from our endocrine clinic. They ranged from total aspermia to occasional necrospermia including the types of cases classified by Moench (4) as 'relative sterility.' The duration of sterility had ranged from 1 to 7 years, the age of the patients from 24 to 40 years. Patients with gonorrhea were excluded from this investigation and the sterility in each case was apparently of the endocrine type.

PROCEDURES

Cases presenting a total aspermia were easy to recognize. However, there were instances in which the female partner was apparently healthy and in which the sperm specimen seemed normal in number and motility, and yet no pregnancy ensued. In such cases we sought other causes for the sterility.

Using the plan formulated by Moench (4) we reorganized our method of sperm examination and instructed our patients to have intercourse with withdrawal and ejaculation into a glass bottle kept nearby in warm water at about 85°F. Spermatozoa will retain their motility best at room temperature, and warmth is more harmful than cold.

[1] Read before the Clinical Society of the Newark Beth Israel Hospital, May 6, 1936.
[2] The extract used in all of these experiments, 'Gamone,' was supplied by E. R. Squibb and Sons.

Our first step was the approximation of the degree of motility and the number of cells. The latter was considered to be within normal limits if the number exceeded 50 per high power field. The average amount of each specimen was approximately 4 cc. We then made stained smears and computed a differential sperm count.

A minimum of 3 successive hundreds was counted and classified as to the proportion of the various abnormalities. In most cases the differences in the 3 groups varied very slightly, usually by less than 5 cells. An average was calculated and this number was accepted as our final result.

A specimen was considered normal only if it fulfilled all 4 of the folowing requirements. *Volume:* The normal specimen should amount to 4 cc. or more. Occasionally after frequently repeated coitus, a concentrated specimen, only about 1 cc. in volume, might be obtained. *Motility:* Normal spermatozoa will remain motile at room temperature for at least 24 hours. *Numbers:* Fifty or more motile spermatozoa per high powered field, constituted a normal sperm count. *Abnormalities:* At least 80 per cent of the spermatozoa must be free of abnormalities.[3] Where from 20 to 25 per cent of the spermatozoa were abnormal, the case was considered one of 'relative afertility.' Where more than 25 per cent of the sperms were abnormal, the entire specimen was classed as abnormal, even though in some instances these patients were able to fertilize. In one of our cases (case 2) this fertilization resulted in repeated abortions.

CASE REPORTS

Case 1. A.S., age 32, had been married for 4 years but had had no children. He denied venereal infection. The clinical duration of sterility was 1 year. Physical examination revealed a well-developed, obese individual. Palpation of the prostate and accessory genital apparatus showed no abnormalities. Repeated spermatic examination, before treatment was instituted 5 months ago, revealed a marked oligospermia and necrospermia with 1 dead sperm per high power field. The B.M.R. was —12 per cent. The Wassermann and Kahn reactions were negative. No abnormalities were found on urinalysis.

Treatment consisted of 25 R.U. of the extract[2] given intramuscularly twice a week. A total of 1000 R.U. was administered over a period of 25 weeks. Spermatic examination at the end of this period revealed 5 actively motile sperms per high power field, with a total of 34 per cent normal sperms. Motility persisted for 10 hours.

Case 2. A. G., age 29, had been married for 5 years but had had no children. He denied venereal infection. This man had 'relative sterility' (4) and numerous impregnations of the female partner had resulted in spontaneous abortions at varied times. A thorough gynecologic examination of the wife failed to disclose any disease or abnormality of the uterus or adnexa. We believed that the disorder existed in the sperm heads. The patient was a well-developed adult male. Palpation of the prostate and accessory genital organs revealed no abnormalities. The B.M.R. was —3 per cent. No abnormalities were found on urinalysis. The Wassermann and Kahn reactions were negative. Spermatic examination, before treatment was instituted, showed 69 per cent normal sperms, and 50 actively motile sperms per high power field. Motility was present for 24 hours.

[3] The types of normal and abnormal spermatozoa used as standards in this classification are illustrated in the 1931 paper by Moench (4).

Treatment consisted of 50 R.U. of the extract twice a week, a total of 950 R.U. being administered over a period of 11 weeks. Spermatic examination at this time revealed 100 actively motile sperms per high power field, with a total of 78 per cent normal sperms. Motility persisted for 40 hours.

Case 3. G. S., age 33, had been married for 7 years but had had no children. He denied venereal infection. Clinical duration of sterile period was 6 years. Physical examination disclosed a well developed male. Palpation of prostate and accessory genital apparatus failed to show any abnormalities. Repeated spermatic examinations before treatment was instituted revealed a total aspermia. The B.M.R. was —4 per cent. No abnormalities were found on examination of the urine. The Wassermann and Kahn reactions were negative.

Treatment consisted of intramuscular injections of 50 R.U. of the extract twice a week. A total of 2000 R.U. was administered over a period of 24 weeks. Spermatic examination at the end of that time revealed 70 actively motile sperms per high power field, with a total normal of 67 per cent. Motility was present for 36 hours.

Case 4. J. M., age 34, had been married for 5 years but had had no children. There was no history or clinical evidence of venereal infection. Physical examination showed a fairly well-developed adult male. Palpation of prostate and accessory genital apparatus disclosed no abnormalities. The patient stated that at no time had he had a nocturnal emission or an ejaculation during coitus, although libido and erection were present. Repeated prostatic examinations before treatment was instituted showed total aspermia and only a minute volume of prostatic secretion could be obtained. The laboratory reports indicated a B.M.R. of +5 per cent, while the Wassermann and Kahn results were negative. No abnormalities were found in an examination of the urine.

Treatment consisted of 125 R.U. injections of follutein a week for a period of 9 months. Examination of prostatic secretions showed no improvement at the end of this time. Then the extract of castrate and menopausal urine was substituted for follutein. Twenty-five R.U. was given twice weekly, making a total of 650 R.U. of the extract over a period of 22 weeks. Examination of the prostatic secretions at this time showed an increase in volume to 1.5 cc., with 12 actively motile sperms per high power field. The proportion of normal sperms was 53 per cent. Motility was present for 10 hours.

Case 5. P. M., age 33, had been married for 4 years but had had no children. He denied ever having had any venereal infection. Clinical duration of sterility was 4 years. Physical examination revealed a well-developed adult male. Palpation of prostate and accessory genital apparatus showed no abnormalities. Examination before treatment was instituted 4 months ago, disclosed an oligospermia. Total volume of semen was 3.2 cc. Motility was present for 4 hours. The proportion of normal sperms was 40 per cent.

The patient received weekly injections of 50 R.U. of the extract for 11 weeks, a total of 575 R.U. being administered. At the end of this time, examination revealed a volume of 4 cc. with 60 per cent normal sperms. Motility persisted for 7½ hours.

Case 6. I. R., age 40, had been married for 8 years but had had no children. There was no evidence, in history or findings, of any venereal disease. The clinical duration of sterility was 6 years. Physical examination disclosed a fairly well developed adult male. On palpation of the prostate and accessory genital apparatus no abnormalities were found. Examination before treatment was instituted revealed a marked oligospermia with a total volume of 1.5 cc. and 44 per cent normal sperms. Motility persisted for 7 hours.

Treatment consisted of intramuscular injections of the extract, 50 R.U. per week for a period of 11 weeks, or a total of 575 R.U. Examination at this time showed an increase in seminal volume from 1.5 to 2 cc. The proportion of normal sperms was 53 per cent. Motility increased in duration from 7 hours to 30 hours.

Case 7. S. S., age 34, had been married for 7 years but had had no children. He denied venereal infection. Clinical duration of sterility was 7 years. Physical examination revealed a poorly developed and undernourished adult male. Palpation of prostate and accessory genital apparatus failed to indicate any abnormalities. Repeated spermatic examinations before treatment was instituted revealed a total aspermia.

Treatment consisted of 50 R.U. of the extract injected intramuscularly twice a week. A total of 1100 R.U. was administered over a period of 15 weeks. Examination at this time showed no clinical improvement. Because of these results, we are now instituting vitamin therapy in conjunction with the extract.

The following cases are presented to demonstrate the comparative value of certain routine office procedures, such as diathermy and prostatic massage alone and in conjunction with the extract of menopausal or castrate urine.

Case 8. C. M., age 34, had been married for 10 years but had had no children. He denied venereal infection. Clinical duration of sterile period was 1 year. Repeated spermatic examination revealed a total aspermia.

Treatment consisted of prostatic massages and diathermy twice weekly over a period of 3 months. No improvement was noted, and 50 R.U. of the extract was injected intramuscularly, once weekly over a period of 12 weeks in conjunction with the above procedures. The total amount of extract was 600 R.U. Spermatic examination at that time showed a total volume of 4½ cc. There were 50 actively motile sperms per high power field, with a total of 62 per cent normal sperms. Motility was present for 20 hours.

Case 9. M. M., age 38, had been married for 8 years but had had no children. He denied venereal infection. Clinical duration of sterile period was 6 months. Repeated spermatic examinations revealed an oligospermia, the majority of sperms being amotile. Treatment consisted of prostatic massage and diathermy twice weekly for a period of 3 months. Since no improvement was noted, 50 R.U. of the extract was injected weekly over a period of 12 weeks in addition to the other procedures. The total amount of extract was 600 R.U. At the end of this period, spermatic examinations showed an increase in total volume to 3.5 cc. with 65 actively motile sperms per high power field. There was a total of 67 per cent normal sperms. Motility was present for 20 hours.

Case 10. A. M., age 24, had been married for 3 years. His wife had never been pregnant. There was no evidence, either in the history or the findings, of any venereal disease. The clinical duration of the sterility was 6 months. Examination revealed an oligospermia with total necrospermia. Treatment consisted of diathermy and prostatic massage for a period of 2 months, with no results. The extract was then used, 50 R.U. weekly for 6 weeks to a total of 300 R.U. At the end of this period, spermatic examination showed an increase in total volume to 2 cc. with 30 sperms per high power field, of which number 6 were actively motile for 12 hours. There was a total of 55 per cent normal sperms. Motility was present for 12 hours.

COMMENT

Since we were working with a principle the clinical reactions of which were unknown, it was imperative that we proceed cautiously. Patients were

selected who showed definite spermatic abnormalities in quantity and quality. Before administration of the extract,[2] a complete spermatic examination was made, and, in addition the B.M.R., Wassermann and Kahn reactions and urinalysis were also studied. We also advised that the frequency of coitus be limited to a feasible minimum.

In our series of 10 cases we have obtained marked improvement in sperm morphology in 9 and no results in 1. Unfortunately, the duration and degree of treatment were variable because of a lack of coöperation on the part of the patients. In some of the cases constitutional reactions resulted from the injections, therefore, gradual desensitization was necessary. Gradually increasing doses were administered twice weekly, intramuscularly, either in the deltoid or gluteal regions until a tolerance level was obtained. The average dose was 50 R.U. twice weekly. Spermatic examinations were repeated each month.

We made careful observations of the effects of intramuscular injections of the extract upon the primary and secondary sex characteristics. Throughout treatment we observed no increase in the size of the prostate or seminal vesicles as shown by palpation nor any gross hypertrophic changes in the testes. The penis showed no change from its original size and there was complete absence of turgescense and engorgement of the scrotal sac. It is also of interest to note that the treatment had no apparent effect upon the secondary sex characteristics, such as hair growth and distribution, girdle obesity, texture of skin, or breast development. Patients 2 and 3 reported a definite increase in libido. In our opinion these observations are of importance since they tend to show that the effect of this follicle-stimulating extract in the male, is purely spermatogenic.

Up to the present we have noticed no deleterious effects following the administration of the extract.

SUMMARY

A follicle-stimulating extract derived from castrate or menopausal urine has been used on 10 male adults suffering from sterility apparently of endocrine origin. In 9 cases the administration of this extract was followed by an increase in the numbers and motility of the spermatozoa. In no case was the improvement sufficient to render the patient fertile during the period of observation here reported.

The average dosage of this extract was 50 R.U. given intramuscularly, twice weekly. The total amount administered varied from 300 to 2000 R.U. In no case was the administration of the extract followed by any deleterious effects.

The author expresses his appreciation to Dr. G. Moench of New York and Dr. J. Morrell of New Brunswick for their assistance in the preparation of this paper.

REFERENCES

1. Smith, P. E. and E. T.: J. Pediat. **5:** 163. 1934.
2. Smith, P. E., E. T. Engle and H. H. Tyndale: Proc. Soc. Exper. Biol. & Med. **21:** 745. 1934.
3. Engle, E. T.: Endocrinology **16:** 506. 1932.
4. Moench, G. L.: Am. J. Obst. & Gynec. **13:** 334. 1927; **19:** 530. 1930; **22:** 199. 1931; **25:** 410. 1933.

THE EFFECT OF SEX HORMONES ON BLOOD-CALCIUM AND INORGANIC BLOOD-PHOSPHATE LEVELS[1, 2]

HUBERT WHATLEY MARLOW AND FRED C. KOCH
From the Department of Physiological Chemistry and Pharmacology, University of Chicago
CHICAGO, ILLINOIS

Changes in the level of blood and serum calcium have been associated from time to time with sex and reproduction. In fowl, pigeons and doves the results are very striking during the egg-laying periods but in mammals the reports are very conflicting and of doubtful significance.

The importance of the calcium intake in fowl during egg production, with normal shell formation, is well established (1, 2, 3, 4, 5, 6) and the hourly fluctuations in the blood-calcium values during laying periods have been beautifully demonstrated in fowl, pigeons and doves (6, 7, 8, 9, 10, 11). Fluctuations in the weights of ovaries, testes and thyroids with seasonal variations in blood-calcium in pigeons and doves were also noted by Reinhardt and Riddle (6). Macowan (10) also observed histological changes in the parathyroids of the laying pullet and showed that parathormone injections caused rises of 2 to 6.5 mg. of Ca per 100 cc. of serum in pullets but not in moulting hens or cockerels.

In the pregnant mammal we also recognize a change in calcium requirement, but the hourly fluctuations in calicum mobilization are not comparable to those existing in avian reproduction, hence changes in levels during pregnancy are not likely to be detected unless the proper calcium intake or absorption or both have been interfered with. These factors and a combination of others which include Ca/P ratio, acid-base balance, vitamin intake and the choice of the analytical method for blood or serum calcium undoubtedly explain the contradictory reports on mammals. Too many experimenters are willing to consider reliable the original Kramer-Tisdall (12) method for Ca, which involves its direct precipitation as oxalate directly from blood serum. The precipitation should be carried out on trichloroacetic-acid filtrates to insure the formation of pure calcium oxalate. Even with this and other improvements, the normal variations in blood-Ca concentration are ± 1 mg. per 100 cc. serum, whereas some investigators have considered a change of ± 0.5 mg. as significant.

A brief résumé of the studies on blood-calcium levels, as a result of gonadectomy, during menstruation and pregnancy and as a result of sex-hormone therapy in mammals, is given below and reveals the contradictory or insignificant results obtained by various investigators.

Gonadectomy. Blinoff (13), Hogben and Charles (14) and Cheymal and Quinquaud (15) find no changes in blood serum after gonadectomy in guinea pigs, rabbits and dogs respectively. These investigators recognize the normal fluctuations

[1] This investigation was supported in part by grants to the University of Chicago by the Rockefeller Foundation and the Committee on Research in Problems of Sex of the National Research Council.
[2] The main results of these investigations were presented at the Sixteenth Annual Meeting of the Association for the Study of Internal Secretions, New Orleans, May 10, 1932.

in blood-calcium values. Decreases are reported by Dalsace and Guillaumine (16) in women, by Suzuki (17) and Okazaki (18) after ovariectomy in rabbits. Rises in blood calcium after gonadectomy were found by Leites (19) in rabbits and by Blauchetiere (20) in women. Leites reports results on only one male and one female by the Clark and Collip (21) modification for blood-serum calcium.

Menstruation. Boynton and Greisheimer (22) report falls in blood calcium during the menstrual or post-menstrual periods in normal women, but that no cyclic variations are observed in dysmenorrhea. These authors used the Clark-Collip modification of the Kramer-Tisdall method for serum Ca and give only the average values on a group of 12 women in the resting, pre-menstrual, menstrual and post-menstrual weeks. Bell (23) also reported lower blood-serum Ca just before menstruation. Widdows (24) found no changes during menstruation. Furthermore, Sherman, Gillett and Pope (25) and Bogert and McKittrick (26) found no change in Ca excretion during menstruation. Frei and Emmerson (27) found no changes in most cows during estrus. In one cow they observed a rise of 1 to 1.7 mg. Ca per 100 cc. prior to each of 3 estrus periods and a fall below normal several days later. The observations by Luckhardt and Goldberg (28) that parathyroidectomized dogs, which have been kept free from tetany by treatment, develop tetany during estrus are of interest and indicate a fall in blood-calcium level.

Pregnancy. Bodo and Liebmann (29), by the use of the frog-heart perfusion method, found no change in the concentration of ionic Ca in human serum as a result of pregnancy. Jansen (30), Mozocco and Moron (31) and Dennis and King (32) could find no significant difference in total serum-calcium in women during pregnancy. Meigs and associates (33) conclude the same in cows. Underhill and Dinnick (34) determined the Ca, Mg, K and Na content of human blood during pregnancy. They used the Kramer and Tisdall methods with trichloroacetic-acid filtrates on the laked blood. Their fluctuations were from 5.5 to 11.1 mg. Ca per 100 cc. of blood during pregnancy and 5.8 to 8.0 in another group of non-pregnant women. Nevertheless, they conclude that pregnancy leads to a rise in blood calcium. Many other investigators find irregular and slight lowering in serum calium during pregnancy in women and cows (35, 36, 37, 38, 39, 40, 41, 42, 43, 44). No systematic attempt seems to have been made to study the same female before and during pregnancy with control of diet. Although in most cases the average values during pregnancy are slightly lower than for other normal females, nevertheless, the differences are of the same order or even less than the normal variations reported by the same investigators. In fact, Stander, Duncan and Sisson (40) regard the differences found by them insignificant and suggest a higher P/Ca ratio during eclampsia, which they associate with a higher inorganic phosphate rather than a lower calcium. Anderson (45), however, considers a value of 9 mg. per 100 cc., or less, as significant, as found in 82 per cent of 44 cases of eclampsia. Intravenous calcium therapy did not appear to relieve the symptoms.

Sex-Hormone Therapy. Mirvish and Bosman (46) call attention to numerous observations which indicate that the ovary plays a rôle in Ca metabolism and emphasize, as illustrations, the change in the skeleton after gonadectomy, the loss of Ca during menstruation, high blood-calcium after menopause and the supposed cure of osteomalacia as a result of ovariotomy. In fact, they assume a physiological antagonism between the parathyroids and the ovaries. They followed the serum-Ca changes in rabbits of both sexes as a result of the administration of crude lipin extracts from various organs and used the Clark and Collip (21) modification for serum Ca but probably precipitated from the serum directly. Their own observations as to the effect of ovariotomy in rabbits indicated no regular change in serum Ca.

Only one estimation was made before operation and 2 to 5 after, even as late as 2 months after the operation. The values varied from 13.9 to 18.0 mg. Ca per 100 cc. serum. A crude alcoholic extract, equivalent to 25 gm. ovarian tissue, injected subcutaneously, they report to lower the blood Ca by 30 per cent in 24 hours, although their normal values varied more than that. Larger doses by mouth gave similar results. No effects were observed from similar extracts prepared from muscle, pancreas, spleen, kidney and brain. Placental extracts also were inactive except in one case when the equivalent of 120 gm. fresh substance was used. The authors claim the same results on normal, pregnant, non-pregnant, mature, immature, ovariectomized and male rabbits. More purified extracts from cows' and pigs' ovaries and from cows' corpus luteum were injected into men and women. In most cases a fall in serum Ca was observed in 12 to 24 hours after one injection. The most striking case gave values of 11.3 and 10.3 mg. per cent before injection and 12, 24, and 48 hours after the injection 10.8, 7.4, and 11.2 mg. per cent, respectively. Their normal blood values varied from 9.6 to 11.6 mg. per 100 cc. serum. They also claim similar effects on male and female rabbits from similar extracts prepared from testis tissue and beef suprarenal cortical tissue, respectively. The results were very irregular, especially when 'more purified' preparations were used. No attempt was made to correlate this activity with estrogenic or other activities.

Reiss and Marx (47) injected rabbits with crude estrogenic material for 13 to 26 days and observed a fall of 1.7 to 3.5 mg. Ca per 100 cc. serum. Before injection the values ranged from 14.1 to 16.1 mg. Manzi (48), in spayed dogs, claims a rise in blood Ca after the injection of 'follicular hormone.' Mathieu and Barnes (49) report that both theelol and theelin lower the blood Ca in parathyroidectomized dogs but not in the normal animal. They found theelol more active than theelin. Dixon (50), however, found no relation between blood-calcium level and estrus, diëstrus, pregnancy, pseudopregnancy or ovarian and anterior pituitary hormone administration. He included dogs, rats and rabbits in his studies. A total dose of 6,000 M.U. of theelol injected in 2 days into young rats had no significant effect on blood Ca.

With anterior pituitary hormone, Cannavo (51) also obtained negative results on dogs, men and rabbits. Hogben and Charles (14), on the basis of very irregular results, conclude that the anterior pituitary pulp may cause a slight fall in blood Ca in rabbits and that following coitus a fall is also observed for some hours. Frei and Emmerson (27), however, consider a 0.5 mg. per cent rise significant following the injection of anterior lobe extracts into a cow in spite of normal fluctuations of 9 to 11 mg. per cent.

The brief review above led us to the conclusion that if the female sex-hormone does influence the blood-calcium level, it would appear that the fowl is the most suitable experimental animal. It was also hoped that more concentrated, more purified and biologically standardized sex-hormone preparations might give more uniform and significant results. We present our data and interpretations on fowl, rats and rabbits below.

Hormone Preparations. A). An estrogenic concentrate (UE) from human pregnancy urine was prepared and biologically standardized for us by Gustavson and D'Amour. This concentrate was diluted with olive oil as needed from time to time.

B). Ovarian Extracts. Pig ovaries were exhaustively extracted with boiling 95 per cent alcohol. This extract was evaporated under diminished pressure

and represents the 'crude extract' of Mirvish and Bosman (46). It is referred to as OE1. This extract is further purified by resolution in a limited volume of 95 per cent alcohol, chilled to remove some fats, phospholipins and cholesterol, evaporated under diminished pressure, dissolved in ether and phos-

TABLE 1. EFFECT OF SEX HORMONES IN FEMALE FOWLS. BLOOD-Ca VALUES IN MG. PER CENT.

Date	Treatment	No. of Fowl						Min.	Max.	Average
		1	2	3	4	5	6			
7/27–30	Normal sample	8.2	6.1	9.2	8.0	—	7.3	6.1	9.2	7.8
7/29–30	Normal sample	11.1	9.4	18.5	7.3	8.8	10.8	7.3	10.8	9.3
7/30–31	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/1		19.4	12.3	13.5	14.8	15.4	15.1	12.3	19.4	15.0
8/1–3	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/4		17.0	12.3	13.3	—	12.5	13.0	12.3	17.1	13.7
8/5	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/6		16.8	12.3	11.1	11.7	11.6	11.3	11.1	16.8	12.5
8/6–7	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/8		16.4	14.3	11.6	12.9	10.0	12.5	11.6	16.4	13.0
8/11	Normal sample	15.7	24.1	13.6	11.2	11.4	14.3	11.2	24.1	15.0
8/13	Normal sample	14.0	21.0	21.8	12.5	11.8	21.1	11.8	21.1	17.0
8/15–16	Injected daily with *3 C.U.* TAn									
8/17		14.5	14.1	13.3	13.3	12.9	14.6	12.9	14.6	13.8
8/17–18	Injected daily with *3 C.U.* TAn									
8/19		12.5	14.7	15.7	14.4	12.7	—	12.5	15.7	14.0
8/19–20	Injected daily with *3 C.U.* TAn									
8/21		14.7	10.5	11.8	12.1	11.6	11.8	10.5	14.7	12.9
8/21–23	Injected daily with *3 C.U.* TAn									
8/24		12.7	10.0	12.5	9.1	9.3	13.3	9.1	13.3	11.2

pholipins precipitated by acetone. The acetone-ether solution was evaporated and the residue again dissolved in a small volume of alcohol, chilled and again taken through the acetone precipitation in ether solution. This product was practically free from phospholipin and cholesterol. It was evaporated and dissolved in olive oil to the desired concentration. An assay for estrogenic activity gave approximately 4 R.U. per cc. of final oil solution. The olive-oil solution

contained the equivalent of 25 gm. fresh tissue per cc. It is referred to as preparation OE.

C). Male-Hormone Preparation. This product was prepared by Dr. T. F. Gallagher from bull testis-tissue and contained 3 capon units per cc. It is referred to as TAn.

Analytical Methods. The blood (1 cc.) was drawn from fowl from a leg vein, from rats by heart puncture under light ether anesthesia and from rabbits by heart puncture without anesthesia. Unless otherwise indicated, trichloroacetic-acid filtrates were prepared from the whole blood. The Ca was determined by the Hauch and Koch (52) micro-modification of the colorimetric method of Roe and Kahn (53). For inorganic phosphate, a micro modification (52) of the Fiske-Subbarow (54) colorimetric method was applied on an aliquot of the trichloroacetic-acid filtrate.

EXPERIMENTAL OBSERVATIONS

Experiments on Fowl. Six young pullets and 6 young cockerels were divided into 3 groups each. Control blood samples were taken shortly after the noon hour, twice with a day intervening. Then, for 2 successive days, 5, 10, and 15 R.U. per 100 gm. body weight of preparation UE were injected into the respective groups. Then followed in sequence a day for taking samples of blood, 3 injection days, sample day, 2 injection days, sample day, 2 injection days, sample day. After 2 days of rest, the same series was repeated but 3 capon units of male-hormone preparation, TAn, was injected instead of the urinary female sex-hormone preparation.

The results are given in detail in tables 1 and 2. For the females, the blood-calcium values prior to any injection varied from 6.1 to 18.1 with an average value of 8.6 mg. per cent. For the males, the range was 6.8 to 11.5 with an average of 8.7. With the exception of the pullet which gave a normal value of 18.5, all the pullets responded with a higher blood calcium after treatment with 5 to 15 R.U.; even the lowest dose gave the maximum change; the range of Ca values for the pullets after the first 2 days' treatment was 12.3 to 19.4 with an average of 15.0 mg. per cent. All the cockerels also responded with a higher blood calcium ranging from 11.8 to 15.9, with an average of 13.3 mg. per cent. Repeated treatment with the same dosage of urinary female sex-hormone did not cause a further rise but rather a somewhat lower value in most cases. A 6-day period without injection did not return the blood calcium to the initial normal value. The cockerels reacted in practically the same way.

The effects from 3 capon units of male hormone daily were less obvious. The tendency was to lower rather than to raise the blood Ca. Inasmuch as the values still were distinctly above the initial figures, these observations are of little value. In fact, a subsequent experiment on pullets and cockerels also indicated that the male-hormone preparation in the dosage employed had no significant effect on the blood-calcium or phosphate levels. The average values indicate a rise in blood calcium and a fall in phosphate, but the individual birds did not react uniformly in the same direction and olive oil alone produced similar irregular changes. In fact, daily bleedings and analyses

TABLE 2. EFFECT OF SEX HORMONES ON MALE FOWLS. BLOOD-CA VALUES IN MG. PER CENT.

Date	Treatment	No. of Fowl						Min.	Max.	Average
		7	8	9	10	11	12			
7/27	Normal sample	9.4	6.8	7.5	7.2	11.5	9.4	6.8	11.5	8.6
7/29	Normal sample	9.4	—	7.2	8.8	9.4	9.4	7.2	9.4	8.8
7/30–31	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/1		12.4	12.5	14.3	12.7	11.8	13.9	11.8	15.9	13.3
8/1–3	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/4		11.4	11.8	11.1	11.2	10.4	10.3	10.3	11.7	11.0
8/4–5	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/6		8.7	11.1	9.6	9.5	10.7	10.3	8.7	11.1	10.0
8/6–7	Injected daily with UE. *R.U.*	*5*	*5*	*10*	*10*	*15*	*15*			
8/8		10.0	12.3	12.5	10.7	12.5	10.0	10.0	12.7	11.4
8/11	Normal sample	11.8	10.0	10.5	11.1	13.3	13.3	10.0	13.3	11.7
8/13	Normal sample	16.7	11.8	11.8	11.3	11.8	11.6	11.3	16.7	12.5
8/14–16	Injected daily with *3 C.U.* TAn									
8/17		10.3	13.1	11.1	12.4	10.0	11.1	10.0	13.1	11.7
8/17–18	Injected daily with *3 C.U.* TAn									
8/19		14.4	12.5	14.0	10.4	12.5	12.5	10.4	14.4	12.7
8/19–20	Injected daily with *3 C.U.* TAn									
8/21		11.4	10.5	10.0	11.0	10.3	11.3	10.0	11.4	10.7
8/21–23	Injected daily with *3 C.U.* TAn									
8/24		11.0	10.0	10.4	13.3	10.0	—	10.0	13.3	11.0

for 2 successive periods of 3 days each also gave fluctuations of 8.1 to 11.6 mg. as average Ca. values for a group of 7 hens and 7 cocks. The fluctuations for an individual bird were, however, much greater in some cases and no sex difference was observed.

The following summer the experiments on young fowl were repeated with estrogenic hormone and with adequate untreated and olive-oil injected controls. The results are given in table 3. The blood-Ca values are so very irregular for treated as well as for untreated fowls that no significance can be attached to the changes observed in the 2 previous experiments. The 2 uninjected fowl fluctuated between blood-Ca values of 12.2 to 20.5 and 10.4 to

16.7 mg. per cent respectively. The 3 olive-oil injected fowl varied from 11.4 to 16.0, 10.5 to 13.1, and 10.6 to 17.4 mg. respectively; after 4 daily injections of 200 R.U. of UE no significant change was observed. Only 1 of the 3 UE-injected fowl responded with a distinctly higher blood Ca than during the preliminary control period. The other 2 showed practically no change after 3 daily injections but after further treatment changed to the preliminary

TABLE 3. ACTION OF URINARY ESTROGENIC HORMONE ON FOWLS. BLOOD CA AND P, IN MG. PER CENT.

Date	Treatment		No. of Fowl							
			Injected with 200 R.U. of UE			Injected with 1 cc. Olive Oil			No Injection	
			157	158	159	154	155	156	152	153
7/18	Normal sample	Ca	26.7	15.7	12.4	14.8	12.9	16.0	20.5	12.9
		P	4.8	4.2	3.6	3.3	4.2	4.8	5.5	3.4
7/20	Normal sample	Ca	22.2	21.3	10.0	16.0	13.1	12.6	16.7	13.2
		P	4.8	4.2	3.8	3.4	3.8	3.5	4.3	4.0
7/21–23	Injected daily									
7/23		Ca	22.2	22.2	20.0	16.0	11.8	13.6	14.8	11.1
		P	4.6	5.0	4.5	3.9	3.2	3.0	3.2	4.0
7/24–25	Injected daily									
7/25		Ca	17.8	21.0	21.8	11.4	10.5	17.4	16.0	10.4
		P	4.8	4.7	4.7	4.6	3.2	3.8	4.3	4.1
7/26–27	Injected daily									
7/27		Ca	14.3	15.4	19.4	12.1	11.7	10.6	14.8	11.9
		P	3.7	4.0	5.3	3.0	3.1	2.9	4.3	4.1
			Injected with 1 cc. Olive Oil			Injected with 200 R.U. of UE			No Injection	
7/28–30	Injected daily									
7/30		Ca	12.9	18.2	22.2	13.4	13.5	14.3	12.2	16.7
		P	4.7	4.6	5.9	3.5	3.3	2.9	3.5	3.3
7/31	Injected									
8/1		Ca	13.2	17.4	19.4	10.8	13.4	11.3	15.4	11.1
		P	4.5	4.5	4.3	3.4	3.4	2.9	2.9	3.6

control level or lower. The inorganic phosphate values varied only slightly. From these observations on such high doses of estrogenic material, we conclude that the urinary estrogenic hormones do not affect the blood Ca in fowl. The rises observed in experiments 1 and 2 no doubt were due to normal fluctuations or to the effect of oil on the original calcium values which were very much lower than in this experiment. In tables 1 and 2, the preliminary control values ranged from 6.1 to 11.5 while in table 3, the range was 10 to 26.7 mg. per cent.

Further attempts to control the normal fluctuations in blood-calcium level in fowl were made. Seven pullets and 8 cockerels were bled 7 times in the course of 10 days. The food intake was controlled and the animals were

caught and placed in small cages some hours before bleeding in an attempt to minimize excitement. The same methods for calcium and inorganic phosphate were used, but special precautions were taken to control the time during which the trichloroacetic acid was in contact with the precipitated protein before separating by centrifuging. The blood-Ca level fluctuated between 7.5 and 14.3 mg. per cent in the females as a group and between 6.5 and 13.8 in

TABLE 4. EFFECT OF BLEEDING AND ESTROGENIC HORMONE ON Ca AND P LEVELS IN ALBINO RATS. VALUES IN MG. PER CENT.

Date	Treatment		Olive Oil Injected			Hormone Injected								
			Female	Males		Females			Males					
			12	3	8	2	9	10	2	4	5	7	9	10
2/7	Uninjected	Ca	7.6	6.1	7.6	—	5.4	9.8	5.7	7.5	8.8	6.6	5.4	5.4
		P	4.7	5.0	3.4	—	4.7	4.0	3.5	4.2	4.5	5.4	4.7	5.5
2/8	Uninjected	Ca	11.1	—	8.3	—	6.7	8.7	6.6	8.9	8.1	—	4.7	—
		P	5.7	—	5.6	—	5.3	4.9	5.0	4.8	5.1	—	5.1	—
2/9	Uninjected	Ca	10.9	6.2	6.2	7.4	8.7	—	8.5	8.5	—	—	7.2	—
		P	3.9	6.0	4.1	4.7	4.7	—	4.8	4.1	4.8	—	4.4	—
2/10	Uninjected	Ca	9.1	—	8.1	—	—	—	—	8.5	7.3	7.8	—	7.7
		P	4.7	—	—	—	—	—	—	4.6	5.1	5.1	—	5.9
2/11	Uninjected	Ca	7.7	6.2	—	9.4	5.8	8.4	6.9	7.6	10.3	6.0	6.5	7.4
		P	5.6	5.8	—	4.9	5.7	5.3	5.1	5.3	6.7	5.1	6.0	6.7
2/12	Uninjected	Ca	7.9	6.9	6.7	7.6	6.0	7.1	9.7	9.5	7.1	7.1	—	9.8
		P	5.6	4.6	—	5.0	5.5	4.6	5.2	6.7	5.9	5.0	—	5.2
2/13–15	Injected		1cc. olive oil daily						10 R.U. of UE daily					
2/16	Uninjected	Ca	10.2	5.7	7.9	9.0	7.0	8.2	7.7	7.9	10.0	8.6	7.1	—
		P	4.0	4.4	3.1	4.8	4.4	5.2	4.1	3.1	4.0	5.2	4.2	—
2/16	Injected		1 cc. olive oil						10 R.U. of UE					
2/17	Uninjected	Ca	8.4	7.9	9.5	7.0	5.7	6.6	7.0	7.0	6.7	6.5	6.7	6.9
		P	5.1	5.3	4.7	3.8	5.5	4.8	5.0	4.5	4.6	5.3	5.3	6.2
2/17	Injected		1 cc. olive oil						10 R.U. of UE					
2/18	Uninjected	Ca	10.5	8.2	9.0	7.1	8.3	6.2	7.0	9.4	7.1	7.0	9.6	7.4
		P	5.0	4.7	4.0	3.1	4.3	3.6	4.3	4.3	4.3	4.6	4.3	5.3

the males. The least variation in individual fowl was 2.1 mg. per cent in females and cockerels each; the maximum variation was 4.7 in an individual pullet and 6.1 in an individual cockerel. Furthermore, after some of these fowl had been allowed to rest for 2 weeks, the blood-Ca values were again determined before and after the 2 daily injections of 10 R.U. of UE and olive oil, respectively. No consistent changes were observed. The fluctuations were in both directions and of the same order as during the long control period. Other fowl of the original group were allowed to recover for 24 days after the preliminary control period. After again determining the normal Ca level, 4 fowl were injected for 2 days with olive oil and 7 for 2 days with 50 R.U. of UE. The olive oil as well as the hormone-injected animals all responded

with slight falls in blood-calcium values. The original values in these fowl were of the order of 8.0 to 11.8 mg. per cent, so that we might have expected a rise as in the first experiment where the original range was 6.1 to 11.5 (tables 1 and 2). Old hens with blood-calcium levels at 13.8 to 24.9 responded equally irregularly to olive oil and urinary estrogenic injections. We are at a loss to explain these discrepancies. However, the normal fluctuations in blood Ca and the irregular results from high doses of urinary estrogenic

TABLE 5. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD CA AND P IN NORMAL AND GONADECTOMIZED ALBINO RATS.

Date	Treatment		Females						Males					
			Normal			Spayed			Normal			Castrated		
			Hormone treated		Control	Hormone treated		Control	Hormone treated		Control	Hormone treated		Control
			31	51	24	2	10	3	11	45	1	2	513	413
4/13	Uninjected	Ca	7.1	6.8	7.9	6.3	5.7	6.3	7.5	6.8	6.6	5.3	4.7	6.3
		P	4.3	4.6	3.8	4.7	3.4	4.0	4.6	4.6	4.3	3.5	3.0	3.3
4/14	Uninjected	Ca	7.7	5.8	6.0	6.0	5.4	5.4	5.7	5.4	7.1	6.3	5.4	6.7
		P	5.0	4.8	4.2	4.8	4.2	3.8	4.8	4.4	4.4	4.1	4.1	3.9
4/15	Uninjected	Ca	6.7	7.8	6.7	6.4	5.8	5.5	7.5	6.5	6.8	—	5.4	7.0
		P	4.4	4.4	3.4	4.3	3.3	3.8	—	4.4	4.0	3.3	3.1	3.5
4/16	Injected with		OE		olive oil	OE		olive oil	OE		olive oil	OE		olive oil
4/17	After 24 hr.	Ca	7.3	7.1	6.4	9.3	8.0	6.5	6.6	6.3	5.8	6.5	9.2	6.8
		P	5.3	5.3	4.6	5.1	4.6	4.0	5.5	5.7	4.4	4.1	4.1	3.9
4/17	After 36 hr.	Ca	10.9	9.1	7.3	6.4	7.9	7.6	8.8	10.0	8.5	7.3	10.0	7.7
		P	5.4	4.9	4.4	4.1	4.9	4.3	5.7	6.5	4.6	4.0	4.7	4.3
4/18		Ca	7.5	9.3	8.4	10.3	8.0	8.2	7.6	8.6	8.8	7.9	9.1	6.1
		P	5.0	5.6	4.4	5.4	4.5	4.4	4.6	6.7	4.8	4.5	3.7	3.8
4/19		Ca	7.8	7.7	7.2	8.8	9.7	7.8	7.3	8.1	7.6	7.3	8.7	6.3
		P	4.9	4.8	3.8	10.0	3.9	4.1	4.0	5.5	4.1	4.0	3.7	3.8
4/20		Ca	7.1	8.3	9.1	—	7.7	7.8	6.8	7.2	9.7	7.1	7.0	6.2
		P	4.5	4.8	4.5	—	4.4	3.8	4.6	5.7	5.1	4.4	4.0	4.6

material lead us to conclude that there is no satisfactory evidence that the estrogenic material affects the level in fowl.

Experiments on Rats. In order to determine the effect of daily heart punctures and light ether anesthesia on the blood-Ca level in rats, 12 animals were bled by heart puncture under light ether anesthesia for 6 successive days and the blood calcium and inorganic phosphate estimated by the methods previously referred to. The greatest fluctuation in blood-calcium values in an animal was 5.7 to 9.7 mg. per cent. In the other 11 rats the fluctuations were less, but the most constant blood Ca in one animal fluctuated between 6.1 and 6.9. Obviously the variations in rats are of the order of 0.7 to 4.0 mg. per cent. The results are given in table 4.

Three of the animals above were next injected with olive oil and the others with 10 R.U. of UE dissolved in olive oil. The effect on blood Ca was very irregular in both groups of animals and the changes were of the order observed prior to the treatment. In fact, the animals which showed the greatest fluctuation before injection also did so after the olive oil and estrogenic hormone injections, respectively. Study of table 4 leads to the conclusion that no significant change in blood Ca was brought about by the injection of estrogenic hormone.

In order to compare our studies with those of Mirvish and Bosman (46), we also studied the effects from a concentrate of the estrogenic fraction from

TABLE 6. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD Ca AND P IN RABBITS.

Date	Treatment		Females				Males		
			Hormone treated		Controls		Hormone Treated		Control
			2	3	1	4	1	4	2
4/29	Uninjected	Ca	11.4	11.0	10.4	11.0	10.5	10.3	12.1
		P	5.9	5.8	5.8	5.7	5.6	6.8	6.2
5/1	Uninjected	Ca	11.4	10.5	10.3	11.1	10.8	10.5	10.8
		P	6.7	5.8	6.1	5.9	5.3	5.8	5.9
5/3	Uninjected	Ca	10.6	10.0	10.3	9.1	11.4	11.1	10.7
		P	5.9	5.8	6.7	5.8	5.8	6.3	5.9
5/4	Injected with		OE		Olive oil		OE		Olive oil
5/5		Ca	13.3	11.9	12.9	10.2	12.3	13.8	11.1
		P	5.6	5.4	5.4	5.7	4.2	5.6	4.6
5/6		Ca	11.9	13.0	18.2	14.8	11.1	15.7	15.7
		P	5.7	4.9	5.0	5.7	5.5	5.5	5.4
5/7		Ca	9.5	12.5	9.8	10.9	10.0	12.9	12.9
		P	5.0	5.5	5.2	5.9	5.3	6.2	5.6

hogs' ovaries. The preparation OE described above was injected for these purposes in doses equivalent to 25 gm. original tissue. The results of these studies on normal and gonadectomized rats are given in table 5. The results on the normal animals confirm the observations with the urinary estrogenic preparation. However, the gonadectomized animals appeared to have somewhat lower blood-Ca values prior to the treatment and the injection of OE equivalent to 25 gm. fresh ovary tended to raise this level. The olive oil did this to a less extent. Before this can be stated to be a fact, more extensive experiments are needed for confirmation.

Experiments on Rabbits. Rabbits were injected with OE in doses equivalent to 25 gm. fresh ovarian tissue and controls were injected with olive oil. In the first experiment the blood drawn by heart puncture without anesthesia was analyzed for Ca and P by the methods used above. The results are given in table 6. The results are contrary to those reported by Mirvish and Bosman

(46). In all cases a rise in blood Ca was obtained and olive oil appeared to be about as efficient as the hormone preparation. The inorganic phosphate tended to become lower in all cases.

In the second experiment on rabbits, the same hormone dosage was used but Ca was estimated in the serum by the Tweedy-Koch (55) modification of the Kramer-Tisdall method (12) and not on trichlor filtrates from whole blood. The results on 2 controls with olive oil and 4 with OE injections are given in table 7. It is obvious that the changes in blood calcium are neither consistent nor significant.

DISCUSSION

Our survey of the literature calls attention to the contradictory claims concerning the relation of blood-calcium level to ovarian activity. It is generally agreed that marked and sudden rises in blood Ca are associated with the

TABLE 7. EFFECT OF WHOLE OVARIAN EXTRACT (OE) ON BLOOD-CA LEVEL IN RABBITS. VALUES IN MG. PER CENT.

Date	Treatment	Females				Males	
		Hormone Treated		Controls		Hormone Treated	Control
		1	4	2	3	2	1
5/19	Uninjected	12.1	15.6	15.4	15.4	14.2	15.0
5/22	Uninjected	15.4	15.2	15.7	16.0	15.2	15.6
5/25	Uninjected	13.6	15.4	14.9	15.4	15.3	15.4
5/25–27	Injected daily with	OE		Olive oil		OE	Olive oil
5/28		15.0	15.0	15.1	15.0	15.1	15.3
5/28–6/1	Injected daily with	OE		Olive oil		OE	Olive oil
6/3		16.1	14.9	16.8	16.4	15.9	16.5

calcification of the egg shell in the dove, pigeon and fowl. How this mobilization of Ca is controlled is not known. From our experiments on fowl, we conclude that we have not been able to associate this rise consistently with an estrogenic activity.

In mammals the need for extra calcium mobilization during pregnancy is also present, but the periodicity of this need is not the same as in birds. The observations on blood-calcium levels in pregnancy have led investigators to claim *a)* that no effect is observed, *b)* that a rise occurs, and *c)* that a fall results. As previously stated, it is probable that proper control of the Ca intake and of other dietary factors will prevent this fall in blood calcium in many cases. It is also conceivable that individuals may possess idiosyncrasies in Ca metabolism and thus yield abnormal values in either direction during pregnancy. To assume that an ovarian hormone controls the level in pregnancy is not well-founded. Nevertheless, Mirvish and Bosman (46) associate ovarian hormone activity with the supposed fall in blood Ca during pregnancy be-

cause they report a fall after the use of their crude ovarian extracts on rabbits and women. We have not been able to confirm these findings on rats or rabbits with urinary or ovarian estrogenic preparations. We have had some suggestions of a rise in blood Ca from such treatment, but the rises are irregular and insignificant as compared with the olive-oil injected controls and with the normal fluctuations observed without injections. Even if we grant that a slightly lower blood Ca is observed in pregnancy, this does not logically lead to the conclusion that the fall is due to an ovarian hormone. The real cause would appear to be the foetus. If the ovarian hormone were to lower the blood Ca without the presence of the embryo, as Mirvish and Bosman (46) claim, the conditions would be very unfavorable for both mother and foetus. It would seem more logical to expect a rise in order to protect the pregnant mother and to aid the foetus. Our observations on the gonadectomized rats appear to lend some support to this possibility because we observed a slight lowering of blood calcium as a result of gonadectomy and a subsequent suggested rise after the injection of the estrogenic material in both sexes. In these studies, the olive-oil injections produced very little and irregular changes in the level. Before these suggestive findings are accepted as proof of hormone action, further studies must be conducted on more animals and with the pure crystalline sex hormones. These were not available when our experiments were carried out.

SUMMARY

The normal blood calcium in fowls fluctuates from 6.1 to 26.7; in rats from 4.7 to 11.1; and in rabbits from 9.1 to 16.0 mg. per cent. Frequent daily bleedings do not appear to be responsible for consistent changes in the level.

A non-crystalline purified estrogenic product prepared from pregnancy urine did not produce a significant and consistent effect on the blood-Ca level in fowls, rats and rabbits. A non-crystalline purified estrogenic preparation from hog ovaries yielded the same results. When gonadectomized rats were studied, the results suggested a slightly lower blood-Ca level than normal and a tendency to a rise after the injection of the ovarian estrogenic preparation. A male-hormone concentrate prepared from bull testis-tissue also produced no change in the level in fowls.

REFERENCES

1. Buckner, G. D., and J. H. Martin: J. Biol. Chem. **41:** 195. 1920.
2. Buckner, G. D., J. H. Martin and A. M. Peter: Am. J. Physiol. **71:** 545. 1925.
3. Buckner, G. D., J. H. Martin and A. M. Peter: Am. J. Physiol. **72:** 255. 1925.
4. Buckner, G. D., J. H. Martin and A. M. Peter: Am. J. Physiol. **72:** 458. 1925.
5. Russell, W. C., and F. C. McDonald: J. Biol. Chem. **84:** 463. 1929.
6. Riddle, O., and W. H. Reinhart: Am. J. Physiol. **76:** 660. 1926.
7. Hughes, J. S., R. W. Titus and B. L. Smits: Science **65:** 264. 1927.
8. Buckner, G. D., J. H. Martin and F. E. Hull: Am. J. Physiol. **93:** 86. 1930.
9. Buckner, G. D., J. H. Martin and W. M. Insko: Am. J. Physiol. **94:** 692. 1930.
10. Macowan, Marion M., Quart. J. Exp. Physiol. **21:** 383. 1932.
11. Charles, Enid, and Lancelot Hogben, Quart. J. Exper. Physiol. **23:** 343. 1933.
12. Kramer, B., and T. F. Tisdall: J. Biol. Chem. **47:** 475. 1921. **48:** 223. 1921.
13. Blinoff, A.: Compt. rend. Soc. de biol. **103:** 187. 1930.
14. Hogben, L., and Enid Charles: J. Exper. Biol. **9:** 139. 1932.
15. Cheymal, J., and A. Quinquaud: Compt. rend. Soc. de biol. **195:** 287. 1932.
16. Dalsace, J., and C. O. Guillaumine: Compt. rend. Soc. de biol. **93:** 1209. 1925.

17. Suzuki, H.: Keijo J. Med. **2:** 332. 1931.
18. Okazaki, T.: Folia pharmacol. japon. **13:** 21. 1932.
19. Leites, S.: Biochem. Ztschr. **150:** 183. 1924.
20. Blauchetiere, A.: Compt. rend. Soc. de biol. **92:** 491. 1925.
21. Clark, E. P., and J. B. Collip: J. Biol. Chem. **63:** 461. 1925.
22. Boynton, R. E., and Esther M. Greisheimer: Proc. Soc. Exper. Biol. & Med. **28:** 907. 1931.
23. Bell, W. B.: Proc. Roy. Soc. Med. **29:** 2. 1908.
24. Widdows, S. T.: Biochem. J. **18:** 555. 1924.
25. Sherman, H. C., L. H. Gillett and H. M. Pope: J. Biol. Chem. **34:** 372. 1918.
26. Bogert, L. J., and E. J. McKittrick: J. Biol. Chem. **54:** 365. 1923.
27. Frei, W., and M. A. Emmerson: Biochem. Ztschr. **226:** 355. 1930.
28. Luckhardt, A. B., and B. Goldberg: J.A.M.A. **80:** 79. 1923.
29. Bodo, R. V., and I. Liebmann: Arch. f. exper. Path. u. Pharmakol. **109:** 178. 1925.
30. Jansen, W. H.: Deutsches Arch. f. klin. Med. **125:** 168. 1918.
31. Mozocco, P., and R. B. Moron: Compt. rend. Soc. de biol. **85:** 692. 1921.
32. Dennis, W., and E. L. King: Am. J. Obst. & Gynec. **7:** 253. 1924.
33. Meigs, E. B., N. R. Blatherwick and C. A. Cary: J. Biol. Chem. **37:** 1. 1919.
34. Underhill, F. P., and Alice Dinnick: J. Biol. Chem. **58:** 133. 1924.
35. Bogert, L. J., and E. D. Plass: J. Biol. Chem. **56:** 291. 1923.
36. Widdows, S. T.: Biochem. J. **17:** 34. 1923.
37. Hess, A. F., and M. J. Matzner: Am. J. Dis. Child. **26:** 284. 1923.
38. Krebs, O. S., and A. P. Briggs: Am. J. Obst. & Gynec. **5:** 67. 1923.
39. Spiegler, R.: Arch. f. Gynäk. **143:** 248. 1930.
40. Stander, H. J., E. E. Duncan and W. E. Sisson: Bull. Johns Hopkins Hosp. **36:** 411. 1925.
41. Adler, M.: Arch. f. Gynäk. **143:** 236. 1930.
42. Mull, J. W., and A. H. Bill: Proc. Soc. Exper. Biol. & Med. **30:** 854. 1933.
43. Godden, W., and W. M. Allcraft: Biochem. J. **26:** 1640. 1932.
44. Pribyl, E.: Klin. Spisy Skoly Zverol. Brno. **9:** 219. 1932.
45. Anderson, D. F.: Brit. J. Exper. Path. **13:** 182. 1932.
46. Mirvish, L., and L. P. Bosman: Quart. J. Exper. Physiol. **18:** 11. 1927. **18:** 29. 1927. Brit. J. Exper. Biol. **6:** 350. 1929.
47. Reiss, M., and K. Marx: Endokrinologie **1:** 181. 1928.
48. Manzi, L., Bull. Soc. ital. biol. sper. **7:** 642. 1932.
49. Mathieu, F., and B. O. Barnes: Am. J. Physiol. **105:** 172. 1933.
50. Dixon, T. F.: Biochem. J. **27:** 410. 1933.
51. Cannavo, L.: Biochem. Ztschr. **245:** 234. 1932.
52. Koch, F. C.: Practical Methods in Biochemistry, William Wood and Company, Baltimore, 1934.
53. Roe, J. H., and B. S. Kahn: J. Biol. Chem. **81:** 1. 1929.
54. Fiske, C. H., and Y. Subbarow: J. Biol. Chem. **66:** 375. 1925.
55. Tweedy, W. R., and F. C. Koch: J. Lab. & Clin. Med. **14:** 1. 1929.

THE EFFECT ON SERUM-CALCIUM AND INORGANIC PHOSPHATE OF FRACTIONS OBTAINED FROM CRUDE OVARIAN EXTRACTS[1]

S. LOUISE HUEY AND H. W. MARLOW
Kansas State College
MANHATTAN, KANSAS

The experimental evidence of the factors and conditions responsible for the changes in blood and serum calcium levels has been reviewed in a previous article by Marlow and Koch (1). This paper discussed the effect of subcutaneous injection of a noncrystalline estrogenic preparation, ovarian extracts and male sex hormone upon the blood-calcium and inorganic-phosphate levels. For experimental animals, fowls (males and females), rats (normal and gonadectomized females, normal males) and normal rabbits (males and females) were used. The results indicate that the levels of serum Ca and inorganic P are not constant but show a daily fluctuation. This is particularly true in fowls, which have a rather wide fluctuation. None of the preparations mentioned above gave significant or consistent effects on the calcium and phosphate levels of the blood.

These findings are not in agreement with Mirvish and Bosman (2), who reported a 30 per cent lowering of serum Ca 24 hours after injection of a crude ovarian extract equivalent to 25 gm. of fresh tissue. They noted that some of the purified extracts did not give such a positive lowering. Marlow and Koch (1) found that the non-crystalline purified estrogenic preparation from hog ovaries did not give positive changes in blood-calcium levels above normal daily fluctuations. It was thought advisable to test each fraction obtained during the process of purification of the crude extract to see whether an impurity in any fraction might contain a calcium-lowering principle.

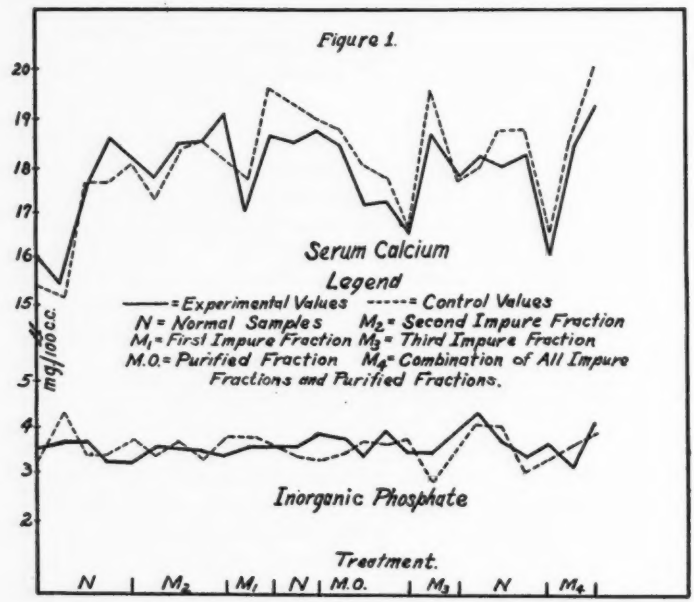
EXPERIMENTAL

Preparation. The preparation of the purified non-crystalline estrogenic extract is described in detail by Marlow and Groetsema (3) and, in its main essentials, in the paper by Marlow and Koch (1). Both papers point out what impurities are removed in the various steps in purification. The different fractions will be designated in this paper as: M_1, composed largely of fats and undoubtedly some phospholipins and cholesterol; M_2, largely phospholipins; M_3, some phospholipins but largely cholesterol; M_4, containing a portion of each of the other impure fractions, together with the purified extract in approximately the same ratio as found in the original crude extract; and MO,

[1] Contribution No. 219, Department of Chemistry.

the final purified extract, containing a large amount of estrogenic material. Each of these impure fractions was dissolved in ether; olive oil was added and the ether evaporated, leaving in each case an olive oil solution of which 1 cc. was equivalent to 25 gm. of fresh ovarian tissue. This amount always caused lowering of serum Ca for Mirvish and Bosman (2). The control animals were injected with olive oil.

Analytical Methods. In order to follow more closely the analytical procedure of Mirvish and Bosman, the serum Ca was determined by the Tweedy-Koch modification (4) of the Kramer-Tisdall method (5). This method, while more critical than many others in which Ca is precipitated directly from



the serum, is not so satisfactory as the method in which Ca is determined on a trichloroacetic acid filtrate. The inorganic P was determined by a micromodification (6) of the Fiske-Subbarow method (7).

Procedure. Male and female rabbits were used, both as controls and as experimental animals. The sample of blood was taken from the marginal ear vein, allowed to clot while in an ice box and centrifuged; the serum thus obtained was used for analysis. These samples were taken at regular times on alternate days. The animals were nearly the same age and size. They were kept under identical conditions of food and environment. They were injected subcutaneously with equivalents of 25 to 50 gm. of fresh tissue, 24 hours before a sample was taken and daily thereafter. The MO (purified extract) was readily absorbed and caused no irritation. On the other hand, all the impure fractions and the mixture M_4 caused more or less irritation. M_1 probably caused less discomfort than M_2, M_3 or M_4. Mirvish and Bosman (2) report a similar result

with their crude extract. This irritation resulted in sores similar in appearance to cholesterol sores.

Before starting injections 5 determinations of Ca and P, covering a period of 11 days, were made on each animal to establish a normal range. The experimental animals exhibited an average range of serum Ca of from 15.5 to 18.6 mg. per cent, while the inorganic phosphate ranged from 3.2 to 3.6 mg. per cent. The control animals showed a calcium range from 15.2 to 18.0 mg. per cent. The phosphate ranged from 3.3. to 4.2 mg. per cent. In table 1 are summarized all of the data from average figures on the serum Ca and on inorganic P. In figure 1, all the average values for the serum Ca and the inorganic P are plotted in the same order as indicated in the table. By following this

TABLE 1. EFFECT OF INJECTION OF THE VARIOUS FRACTIONS UPON SERUM CALCIUM AND INORGANIC PHOSPHORUS. VALUES IN MG. PER 100 CC.

Treatment	Calcium Range		Inorganic Phosphate Range	
	Experimentals	Controls	Experimentals	Controls
1 cc. olive oil (Normals)	15.5–18.6	15.2–18.0	3.2–3.6	3.3–4.2
2 cc. M_2	17.7–18.5		3.5–3.5	
2 cc. olive oil		17.3–18.5		3.4–3.8
2 cc. M_1	17.0–19.2		3.4–3.5	
2 cc. olive oil		18.2–19.7		3.7–3.8
1 cc. olive oil (Normals)	18.5–18.8	19.0–19.3	3.6–3.9	3.3–3.4
1 cc. MO.	17.2–18.4		3.4–3.9	
1 cc. olive oil		17.8–18.8		3.5–3.6
2 cc. M_3	16.4–18.7		3.4–3.8	
2 cc. olive oil		16.6–19.6		2.8–3.7
1 cc. olive oil (Normals)	17.9–18.3	17.9–18.8	3.4–4.3	3.0–4.1
2 cc. M_4	16.0–19.2		3.2–4.0	
2 cc. olive oil		16.4–19.9		3.3–3.8

graph the average values, fluctuations and similarity of fluctuations of the experimentals and controls during the various treatments can be observed.

CONCLUSIONS

The serum-calcium and inorganic-phosphate levels of rabbits are not constant but fluctuate from time to time.

The pure non-crystalline extract, MO, is as readily absorbed as olive oil and without irritation, while the impure fractions, M_1, M_2 and M_3 and the mixture M_4, are irritating and not so readily absorbed. Control animals receiving only olive oil show as great variation in serum Ca and inorganic P as the experimentals on any of the impure fractions. The results on the graph show a like daily fluctuation, as though there were some common cause which has not as yet been determined.

It may therefore be pointed out that neither any of the fractions eliminated during the preparation of the purified extract, nor the purified extract, nor

a combination of all of these, causes a definite and consistent change in the serum Ca and inorganic P.

Part of the data for this paper were taken from an unpublished thesis submitted by one of us (S.L.H.) in partial fulfillment of the requirements for the degree of Master of Science at Kansas State College. The authors wish to thank Dr. F. C. Koch of the Department of Biochemistry of the University of Chicago for his aid in furnishing the ovaries and the materials used in this research.

REFERENCES

1. Marlow, H. W., and F. C. Koch: Endocrinology **21:** 72. 1937.
2. Mirvish, L., and L. P. Bosman: Quart. J. Exper. Physiol. **18:** 11. 1927. Ibid. **18:** 29. 1927. Brit. J. Exper. Biol. **6:** 350. 1929.
3. Marlow, H. W., and Frederick Groetsema: Endocrinology **19:** 41. 1935.
4. Tweedy, W. R., and F. C. Koch: J. Lab. & Clin. Med. **14:** 1. 1929.
5. Kramer, B., and T. F. Tisdall: J. Biol. Chem. **47:** 475. 1921. Ibid. **48:** 223. 1921.
6. Koch, F. C.: Practical Methods in Biochemistry. William Wood and Co., Baltimore, 1934.
7. Fiske, C. H., and Y. Subbarow: J. Biol. Chem. **66:** 375. 1925.

THE QUANTITATIVE DETERMINATION OF THE GONADOTROPIC MATERIAL OF URINE OF WOMEN AFTER CASTRATION AND THE MENOPAUSE AND OF NORMAL MEN

PHILIP A. KATZMAN
From the Laboratory of Biological Chemistry, St. Louis University School of Medicine
ST. LOUIS, MISSOURI

During the past few years we have been intermittently engaged in the development of methods for the quantitative estimation of small amounts of gonadotropic substances. We have reported that small quantities of the gonadotropic substances of urine of pregnant women and of unfractionated anterior lobe extracts which were added to boiled urine of normal men were satisfactorily recovered by adsorption on benzoic acid or preciptation with tungstic acid (1, 2). In that work the assay was based on the induction of vaginal opening and cornification in immature rats. Since the active material from these sources was successfully recovered by these methods, we assumed that the gonad-stimulating substances of various urines could likewise be determined.

However, other investigators who were using the alcohol precipitation method and a different method of assay found that the benzoic acid method gave low results when applied to urine obtained from women after castration or the menopause (H. M. Evans, O. W. Smith, L. Levin, personal communications; Frank, Salmon and Friedman, 3). We have, therefore, investigated this phase of the problem by comparing the yields obtained on the same urine samples by the different methods of extraction, employing two different criteria of activity.

EXPERIMENTAL

Recovery of the Gonadotropic Material of the Urine of Pregnancy and of Anterior Lobe Extracts. First, we reinvestigated the effectiveness of benzoic and tungstic acids in the recovery of small amounts of the gonadotropic substances of urine of pregnancy and of the anterior pituitary when these are added to boiled urine. The procedures were carried out as described in our previous publication (2), the acetone-dried precipitates being extracted with dilute NaOH and neutralized with dilute acetic acid. The anterior lobe extracts, before and after recovery, were assayed both on the basis of vaginal estrus and a 100 per cent increase in the weight of the ovaries. The assay of the extracts of the urine of pregnancy was based only on the former, since small amounts of this material have very little effect on the weight of the ovaries.

The results obtained agreed essentially with our earlier findings. The material from the urine of pregnancy was completely recovered in every case

TABLE 1. AVERAGE YIELDS FROM MENOPAUSE AND CASTRATE URINES BY ALCOHOL PRECIPITATION AND TUNGSTIC AND BENZOIC ACID ADSORPTION.[1]

Urine Samples	Assay Method	Alcohol Precipitation	Tungstic acid—NaOH	Benzoic Acid	Tungstic Acid	
					$Ba(OH)_2$	$BaCO_3$
10 samples from castrates	Estrus	% 100	% 103.5	% 47	% 94	% 91
	Double ovarian weight	100	98.0	<30	85[2]	80[2]
10 samples after the menopause	Estrus	100	95.0	56.0	105	92
	Double ovarian weight	100	60	<35	93[3]	<60[3]

[1] A total of 5 to 10 animals were used for the assay of each extract.
[2] Determinations made on 12 samples of castrate urine.
[3] Determinations made on 4 samples of menopause urine.

by both tungstic and benzoic acids. With the anterior lobe extracts satisfactory recoveries were made by both extraction methods using both methods of assay. The tungstic acid method was superior, however, in several instances yielding somewhat more than a 100 per cent recovery. This may have been due to an augmentation such as reported by Fevold et al. (4), Maxwell (5), Saunders and Cole (6) and Hellbaum (7).

Recovery of the Gonadotropic Material from the Urine of Women after Castration and the Menopause. The gonadotropic content of each urine sample was measured by precipitation with alcohol and tungstic acid and by benzoic acid adsorption. In the tungstic acid procedure it is advisable to centrifuge the tungstate precipitate rather than to allow it to settle out. In each method the acetone-washed and dried precipitate was dissolved or extracted with dilute NaOH, the extract neutralized with acetic acid and assayed on 21-day-old female rats. The animals were examined for vaginal estrus and 100 to 120 hours after the injections were started were killed and the ovaries dissected and weighed.

The average results obtained on 10 samples each of urine after the menopause and after castration are given in table 1. The assays of the extracts prepared by precipitation with alcohol were taken as representing 100 per cent of the activity of the urine. Using vaginal estrus as the criterion of activity, the tungstic acid NaOH method gave complete recovery for both types of urine. When the activity was based on doubling the weight of the ovaries,

TABLE 2. COMPARISON OF YIELDS OBTAINED BY ALCOHOL, TUNGSTIC ACID AND TANNIC ACID FROM CASTRATE URINE.

Urine	Rat Units per Liter (double ovarian weight)		
	Alcohol	Tungstic Acid	Tannic Acid
K11	14.0	13.2	10.2
K12	14.0	16.6	13.2
K13	9.5	11.1	11.0
K14	13.3	9.5	13.3
K15	<7.5	7.4	7.4

this method gave satisfactory results with the urine of castrated women but rather low results with urine after the menopause. The benzoic acid method yielded extracts possessing low activity for both types of urine.

In order to eliminate as far as possible the variations due to the test animals, determinations were carried out on another series of urine of castrates using 3 to 10 test animals for each dosage of every preparation. In this series alcohol, tungstic acid and tannic acid were used. The tannic acid was used according to the procedure described by Levin (8).[1] The data recorded in table 2 are based on a 100 per cent increase in the weight of the ovaries. As is shown, the tungstic acid method gave yields which were equal to or greater than those obtained by the other methods in all but 1 instance (K14). Because of an inadequate supply of this sample the reason for this discrepancy could not be ascertained.

The active substance of the urine of castrates may be successfully liberated from the tungstic acid precipitate by means of either $BaCO_3$ or $Ba(OH)_2$ while for urine after the menopause only $Ba(OH)_2$ is satisfactory (table 1). In this manner these reagents give results superior to those obtained with brucine. Solid $BaCO_3$ is mixed with the acetone-dried tungstic acid precipitate and the mixture is suspended in the desired amount of water. Enough $BaCO_3$ is added to make the mixture neutral to litmus paper. After mixing thoroughly until all small lumps are powdered, the mixture is centrifuged and injected. If $Ba(OH)_2$ is used, a 1 per cent solution is added to the dry tungstic acid precipitate until the mixture becomes just alkaline to phenolphthalein. All lumps are carefully powdered and the mixture is centrifuged. Excess Ba is removed by adding Na_2SO_4 solution and centrifuging. By these means fairly good recoveries are made and purification is effected. The tungstic acid precipitate of urine after the menopause treated with $Ba(OH)_2$ gave better yields than were obtained with NaOH. A small loss was incurred in these determinations since the precipitates were not reextracted. However, this was not significant because the bulk of the precipitate was small in comparison to the volume of the extract.

As can be seen from table 1, benzoic acid recovers from 50-60 per cent of the activity of urine obtained from women after the menopause or castration, when the assay is based upon opening of the vagina and cornification. Less than 30 per cent is obtained when the criterion is a 100 per cent increase in the weight of the ovaries. Since this method yields satisfactory results with the gonadotropic material of the anterior pituitary and of the urine of pregnancy, it was of interest to attempt to determine why this was not the case with the active material of the urine after the menopause and castration.

First, extracts of urine after the menopause prepared by precipitation with alcohol were added to boiled urine of normal men in the usual manner and recovery was attempted by the benzoic acid method. Four determinations were made in which the quantity of added material varied from 3.7 to 8.3 R.U.,

[1] The urine is acidified to pH 5 with acetic acid. To each liter of urine is added 20 cc. of an aqueous 10 per cent solution of tannic acid. The mixture is allowed to stand in the cold room for an hour or two with occasional stirring. The precipitate is collected by centrifugation, washed with acetone, dried, and extracted with dilute NaOH.

based on a 100 per cent increase of ovarian weight. In no case was more than 40 per cent recovered. The added material was satisfactorily recovered by the alcohol precipitation method if benzoic acid was not added.

Then, the reagents used in the method were studied individually for destructive or inhibitory action. In these experiments, the reagent under investigation was added to the urine and an extract then prepared by the alcohol method. The activity of this extract was compared with that of a similar extract in which the particular reagent was omitted. Table 3 shows that the low activity is due to the added benzoic acid, the acetone and acetic acid having no effect.

TABLE 3. EFFECT OF REAGENTS USED IN THE BENZOIC ACID METHOD ON THE GONADOTROPIC MATERIAL OF MENOPAUSE AND CASTRATE URINE.

Urine Samples	Assay Method	Alcohol Precipitation	
		Without Reagent	With Reagent
Reagent—acetic acid			
2 Menopause and 2 Castrate	Estrus	100%	>90%
	Double ovarian weight	100%	102%
Reagent—acetone			
2 Menopause and 4 Castrate	Estrus	100%	104%
	Double ovarian weight	100%	95%
Reagent—acetone+benzoic acid			
2 Menopause and 4 Castrate	Estrus	100%	71%
	Double ovarian weight	100%	<60%

Furthermore, if the benzoic acid method is so modified that the benzoic acid is obtained by adding sodium benzoate to the urine and acidifying carefully with HCl, the yield is not improved. Precipitation with alcohol after benzoic acid adsorption, yields relatively inactive extracts. Whenever precipitation with alcohol is carried out in the presence of benzoic acid (15-25 gm. per liter of urine) the same low yields are obtained as by benzoic acid adsorption. Combining the fraction which is adsorbed on benzoic acid with the fraction which is precipitated by alcohol after benzoic acid adsorption, results in no increased activity. Therefore the low values which were obtained do not appear to be the result of a separation of factors but rather to an inactivation which might have been caused by the acidity of the benzoic acid.

It is interesting to note that the activity of the benzoic acid preparations is much higher when assayed on the basis of vaginal estrus than when based on a weight increase of the ovaries. From this it would seem that the benzoic acid modifies the active material of menopause and castrate urines to produce a product which retains a considerable portion of its activity toward inducing

vaginal opening and cornification but which has lost its ability to increase the size of the ovaries. Maxwell and Bischoff (9) and Loeb and coworkers (10) have also noted altered responses induced by chemical treatment.

With the urine of normal individuals the situation is apparently quite different. Studying the daily excretion of gonadotropic material by normal adult men and women we found no significant differences between the tungstic acid and benzoic acid methods (2). Vaginal opening and estrus was the basis of assay in this work. Frank and Salmon (11) obtained satisfactory results in the determination of the daily excretion of this factor by normal women, using the benzoic acid method. In the present investigation an attempt was made to compare the yields of gonadotropic material obtained from the urine of normal men by the various methods. The data are shown in table 4.

The gonad-stimulating content of each of 10 pooled urine samples was

TABLE 4. YIELDS OF GONADOTROPIC MATERIAL OF URINE OF NORMAL MEN. VALUES IN RAT UNITS PER LITER.

Urine	Alcohol	Tungstic Acid			Benzoic acid	Sodium Benzoate	Tannic acid
		NaOH	$Ba(OH)_2$	Brucine			
N1	4.6	2.0	2.4	—	1.4	<1.4	2.0
N2	<4.7[1]	<1.6[1]	<1.9[1]	—	1.3	<1.2	1.8
N3	2.4	1.9	1.8	—	2.1	<1.6	2.5
N4	<3.0[1]	<1.6[1]	<1.6[1]	—	1.6	2.2	1.7
N5	<2.6[2]	<1.8[1]	<1.6[1]	1.6	1.5	1.5	1.6
N6	2.2[2]	Toxic	—	—	1.4	—	1.5
N7	<2.2[2]	Toxic	—	—	1.4	—	1.4
N8	<2.2[2]	—	—	1.2	<1.1	—	2.1
N9	<2.1[2]	—	—	1.7	<1.3	—	1.7
N10	1.9[2]	—	—	1.5	1.5	—	1.5

[1] Toxic in larger doses.
[2] Modified alcohol-method.

determined by the methods shown in table 4. Tungstic, benzoic and tannic acids were used in the same manner as previously described. When sodium benzoate was used, 15 to 20 gm. were added for each liter of urine, followed by the careful addition of HCl until pH 4 to 4.5 was reached. The precipitated benzoic acid was treated exactly as in the benzoic acid procedure.

In most instances the extracts prepared by the alcohol method were not tolerated by the test animals. In order to decrease the toxicity the procedure was modified as follows. The material precipitated by alcohol was extracted 3 times with small volumes of 40 per cent alcohol and 10 volumes of acetone added to the combined alcoholic extract. A little sodium acetate solution was added to facilitate precipitation. The precipitate was collected by centrifugation, washed with acetone, dried by means of suction and dissolved in distilled water.

The difficulties involved in obtaining accurate assays of extracts of this type are, of course, apparent. The amount of active material is exceedingly small and difficult to separate from the large quantities of interfering substances without incurring appreciable losses. In the present investigation, those animals which showed ill effects as a result of the injections were not included

in the assay. However, the contaminating material undoubtedly alters the response to the active principle even though it may appear to be tolerated by the test animals.

The assay of the extracts was conducted according to the procedure described earlier in this report. At least 2 and, in most instances, 3 or 4, animals were used for each dosage of every preparation. Since an appreciable increase in the size of the ovaries was not induced by any extract in the amounts administered, the values in table 4 are based on vaginal opening and cornification.

In only 1 instance (N1) did the alcohol method give a significantly greater yield than that obtained by the other methods. Although the exact activity of the extracts prepared by the alcohol procedure was not determined in most cases, it is evident that the yields obtained by this process could not have exceeded those obtained by the other methods to any appreciable extent. Of course, it should be remembered that since the method involving the use of alcohol was modified to diminish the toxicity the comparison may not be entirely fair.

The active material of the extracts prepared by the tungstic acid NaOH and $Ba(OH)_2$ methods was usually masked by toxic material, for in those instances in which the toxicity was not great, satisfactory yields were obtained. All of the preparations obtained by the tungstic acid-brucine procedure were satisfactory with respect to yield and toxicity. The sodium benzoate failed to give adequate yields although the extracts obtained by its use were not toxic. Perhaps the acidity was excessive in this procedure.

The extracts prepared by the tannic acid method although very crude were fairly well tolerated by the animals. Extracts obtained by means of the benzoic acid process were the least toxic, being clear and only slightly colored. The yields obtained by both methods compare favorably with the exception of 2 instances (N8 and N9).

It seems, therefore, that the extraction of the gonadotropic material of the urine of normal men may be accomplished by means of tungstic, benzoic or tannic acid. For this reason and because ovarian enlargement was not obtained it would seem that this material differs from that found in urine of women after castration or the menopause. However, it is possible that the true action of the exceedingly small quantities of active material is altered or masked by the inert and toxic substances present in the extracts.

SUMMARY

Data are presented on the comparison of yields of gonadotropic material obtained from the urine of women after castration and the menopause by precipitation with alcohol, tungstic and tannic acids and adsorption on benzoic acid. Contact of these urines with benzoic acid causes a diminished and altered activity. Satisfactory results may be obtained by means of tungstic acid, benzoic acid or tannic acid when urine of normal men is used.

The writer is deeply indebted to Dr. E. A. Doisy for his advice and suggestions and to Miss Corinne Dewes for her assistance with the assays.

REFERENCES

1. Katzman, P. A., and E. A. Doisy: Proc. Soc. Exper. Biol. & Med. **30:** 1188. 1933.
2. Katzman, P. A., and E. A. Doisy: J. Biol. Chem. **106:** 125. 1934.
3. Frank, R. T., U. J. Salmon, and R. Friedman: Proc. Soc. Exper. Biol. & Med. **32:** 1666. 1935.
4. Fevold, H. L., F. L. Hisaw, A. Hellbaum, and R. Hertz: Am. J. Physiol. **104:** 710. 1933.
5. Maxwell, L. C.: Am. J. Physiol. **110:** 458. 1934.
6. Saunders, E. J., and H. H. Cole: Proc. Soc. Exper. Biol. & Med. **33:** 504. 1936.
7. Hellbaum, A. A.: Proc. Soc. Exper. Biol. & Med. **33:** 568. 1936.
8. Levin, L., Twenty-ninth Annual Meeting of the American Society of Biological Chemists, (1935).
9. Maxwell, L. C., and F. Bischoff: J. Biol. Chem. **112:** 215. 1935.
10. Loeb, L., W. C. Anderson, J. Saxton, S. J. Hayward, and A. A. Kippen: Science **82:** 331. 1935.
11. Frank, R. T., and U. J. Salmon: Proc. Soc. Exper. Biol. & Med. **32:** 1237. 1935.

THE EFFECT OF ANTERIOR PITUITARY-LIKE HORMONE ON THE BLOOD PICTURE IN RABBITS

DAVID WILSON
Department of Anatomy, the Ohio State University
COLUMBUS, OHIO

Clinical evidence indicates that the processes of hemopoiesis and discharge of young cells into the circulating blood are controlled by agents in the circulating blood itself. Hubble (1) expresses the view that secretions of the endocrine glands are concerned in this process. In a review of the literature he finds both experimental and clinical evidence indicating that the thyroid hormone stimulates the production of red blood cells and lymphocytes and depresses the output of granulocytes; the adrenal cortical hormone stimulates the production of granulocytes and probably also of red blood cells, while the basophil cells of the anterior lobe of the pituitary stimulates all types of circulating cells.

Damble (2) and McCullagh and Dunlap (3) have given both clinical and experimental proof of the relation of the thyroid to hemopoiesis. Rogoff and Stewart (4) demonstrated an increase of 50 to 100 per cent in the red blood cells following the injection of cortico-adrenal extract. This increase they believed to be due to dehydration alone. Corey and Briton (5) produced a leucopenia resembling agranulocytic angina by adrenalectomy and remedied the condition by the injection of cortico-adrenal extract. Fox and Whitehead (6) found no change in the white blood counts with injections of cortico-adrenal extract in normal animals.

Adrenalin causes a leucocytosis with an increase taking place in all types of cells and a relative increase of lymphocytes. This reaction is believed to be not the result of a specific hemopoietic stimulus but a physiological reaction due to the contraction of the smooth muscle of the spleen and lymph nodes. A similar response has been elicited by exercise (7). The leucocytes increased from 15 to 40 per cent and the return to normal took place in 30 minutes.

Little is known of the effect of the sex hormones on the hemopoietic organs. A sex difference is known to exist between the male and female members of a species but other than hormonal factors may be present as a basis of explanation. The leucocytosis and increase in wandering connective tissue cells, demonstrated in the uterus and elsewhere during pregnancy, tends to indicate a stimulus of the bone marrow and connective tissues. Likewise, the injection of theelin in normal female rabbits has been shown to cause an increase of phagocytic cells in the uterus (8). But, in a case of leukemia, a superimposed pregnancy with normal delivery did not aggravate the blood picture (9). Testicular extracts increase the red blood cell fragility (10).

Posterior pituitary extract (British Pharmacopeia) injected alternately with an acetone picric acid extract of the posterior pituitary has been demonstrated to produce a macrocytic anaemia in rabbits. The anaemia develops suddenly, by the fourth or fifth day, and the red blood cells drop to as low as one million. An associated

leukocytosis to 50,000 also occurs. The interpretation of this reaction is hard to make since the injections likewise cause hemorrhagic ulcerations of the acid secretive portion of the stomach (11).

Geist and Spiellman (12) studied the effects of antuitrin-S injections on the blood of clinical patients and found no significant changes 24 hours after the injection. Stockinger and Berchtold (13) however found a transient leucocytosis when counts were made in the few hours following the injections.

The experiments reported here were undertaken in an attempt to study the effects of anterior pituitary-like hormone on the blood in regard to a possible relation to the increase of leucocytes occurring in pregnancy and blood disorders and to the control of normal hemopoiesis.

In our series of experiments, 193 injections were made into the marginal vein of virgin female rabbits weighing between 4 and 8 lb. Baseline counts of 10 to 15 readings were made over a period of 1 to 1½ months. By this time the rabbits were well acclimated to their surroundings and to consider-

TABLE 1.

Rabbit No.	Number of Injections	Baseline of White Blood Cells	Highest Value Recorded
A	11	6– 7,000	20,050
B	12	5– 7,000	14,000
C	30	9–10,000	34,000
D	30	9–11,000	33,000
E	25	7– 8,000	16,000
F	36	14–20,000	60,000
G	6	7– 9,000	26,000
H	10	7– 9,000	24,000
I	13	6– 8,000	24,000
J	8	7–12,000	17,000
K	12	8–12,000	38,000

able handling. Cognizance was given to the effects of mild provocation in handling (14) and to the diurnal changes (15). The blood cell counts were made immediately preceding the injections, at 3 to 8 hours afterwards and again on the following day. The interval for injections was governed by the reaction of the blood. In general an injection was made every 2 or 3 days. One hundred R.U. was given as the standard dose. Both antuitrin-S (Parke-Davis) and preparations extracted in our own laboratory by the alcohol precipitation method were used. The reaction was similar in all cases.

Each injection was made in the marginal vein from which blood for the base reading had previously been drawn. Successive readings were then taken from the opposite ear. Alternate ears were used for subsequent injections. Automatic Trenner blood pipettes were used and total counts were made in a Levy counting chamber. Differential counts were made by the supravital method and Wright and reticulocyte slides were made in the order mentioned.

After each injection a marked leucocytosis occurred reaching its height in 5 to 8 hours and maintaining a relative high level for 48 to 72 hours. The base line and highest readings in each case are given in table 1.

The highest reading was usually found to follow the first injection but occasionally higher values were reached on the second or even a later injection. The height was governed in some respects by the time interval allowed between injections. When an interval of 10 days to 2 weeks was allowed between injections the leucocytosis was similar in extent following each injection. Less and less reaction was elicited when injections were given at shorter intervals of every 2 days. It would appear from this that a certain percentage of tolerance was established to frequent injections. If continued long enough a time was reached when the injections elicited no increase in leucocytes. The whole process with an initial high rise could however be elicited again after an inter-

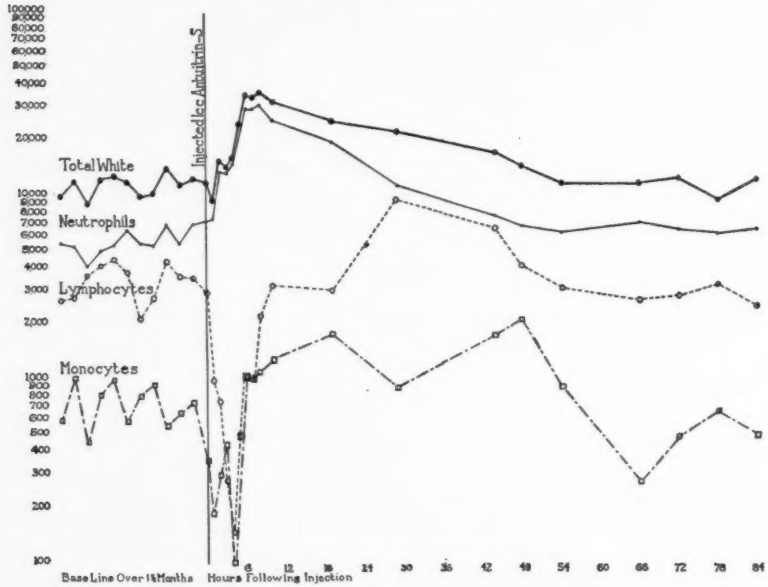


Fig. 1.

val of 2-weeks' rest. This may have some bearing on Collip's theory of antihormonal control (16).

In those cases in which the blood picture could be followed at shorter intervals of every 1 or 2 hours, the course of the blood changes was similar in all cases and characteristic. The other readings were fitted into the curve according to the time interval after the injection. A slight drop in the total white blood cells occurred in the first or second hour following the injection. Thereafter the total count rose rapidly and remained at a relatively high level for 48 to 72 hours, usually returning to normal in 4 to 5 days. The neutrophils showed a greater increase both in relative and absolute numbers, usually totaling 85 to 95 per cent at the height of the reaction. The other elements, lymphocytes, eosinophils, basophils and especially the monocytes, were correspondingly depressed. The peak of the reaction from a single injection both

in different rabbits and in the same rabbit on different injections varied somewhat in its interval following the injection. Occasionally no rise was recorded in the first day but was found 24 hours later; usually, however, the peak of the reaction was found 5 to 8 hours following the injection.

The differential changes were likewise characteristic. The neutrophils after reaching the peak of 85 to 95 per cent in 5 to 8 hours gradually declined to their normal level while the percentage of lymphocytes correspondingly increased. In 24 to 48 hours the percentages of neutrophils and lymphocytes approached each other. A typical reading was: neutrophils 42, lymphocytes 45, eosinophils 5, basophils 4 and monocytes 4 per cent, as compared with the normal values of neutrophils 65, lymphocytes 26, eosinophils 4, basophils 3 and monocytes 2 per cent. For the next 24 to 48 hours the lymphocytes, basophils, eosinophils and monocytes increased above their normal level and the neutrophils were correspondingly further decreased. The lymphocytes and, later, monocytes during this period showed the predominant increase. The lymphocytes then the monocytes returned to normal. The reaction is illustrated graphically by counts on rabbit K following a single injection (fig. 1).

Many young forms were occasionally found in the blood during the early stages of the reaction. This probably indicates bone marrow stimulation similar to that of a toxic reaction. The latent time interval however following the injection might suggest specific organ stimulation, presumably through the ovaries.

The red blood cells showed no change.

DISCUSSION

That hormones play some part in the control of hemopoiesis either directly or indirectly can be postulated from the experimental and clinical evidence now on record. Our results show that injections of antuitrin-S or APL substances cause a marked leucocytosis with no change in the erythrocytes. If the injections are continued over a long period of time and at relatively short intervals they become ineffectual in the production of a leucocytosis. Some substance appears to be developed in the blood of the injected rabbits which neutralizes the effect and inhibits the leucocytic stimulation. This inhibitory effect is of short duration since the initial response can be elicited if 10 days to 2 weeks are allowed to elapse between injections. This reaction may be of an antihormone nature. It is possible that the leucocytic response is due to some constituent of the extract other than the hormone itself—possibly of protein nature. The nature of the reaction is unknown at present.

SUMMARY

Injections of APL hormone extracts, such as antuitrin-S, caused a marked leucocytosis in normal virgin female rabbits within 5 to 8 hours following the injection. The red cell count remained unaltered. Repeated injections over a long period and at short intervals became ineffectual in producing a leucocytosis.

Appreciation is extended to Dr. R. A. Knouff for his advice and interest in the completion of this paper.

REFERENCES

1. Hubble, D.: Lancet **225:** 113. 1933.
2. Damble, K.: Klin. Wchnschr. **12:** 497. 1933.
3. McCullagh, E. P., and J. A. Dunlap: J. Lab. & Clin. Med. **17:** 1060. 1932.
4. Rogoff, J. M., and G. N. Stewart: Am. J. Physiol. **78:** 711. 1926.
5. Corey, E. L., and S. W. Britton: Am. J. Physiol. **102:** 699. 1932.
6. Fox, C. A., and R. W. Whitehead: Proc. Soc. Exper. Biol. & Med. **32:** 756. 1935.
7. Martin, H. E.: J. Physiol. **75:** 113. 1932.
8. Fluhman, C. F.: Am. J. Obst. & Gynec. **24:** 654. 1931.
9. Kaplan, I. I., and J. E. Connery: Am. J. M. Sc. **183:** 209. 1932.
10. Favelli, G.: J. Exper. Med. **54:** 197. 1931.
11. Dodds, E. C., and R. L. Noble: Nature **135:** 788. 1935.
12. Geist, S. H., and Spiellman, F.: Proc. Soc. Exper. Biol. & Med. **32:** 353. 1934.
13. Stockinger, W., and F. Berchtold: Ztschr. f. d. ges. exper. Med. **75:** 827. 1931.
14. Schattenberg, H. J., and W. H. Harris: Proc. Soc. Exper. Biol. & Med. **29:** 269. 1931.
15. Sabin, F. R., R. S. Cunningham, C. A. Doan and J. A. Kindwall: Bull. Johns Hopkins Hosp. **37:** 14. 1925.
16. Collip, J. B.: Ann. Int. Med. **8:** 10. 1934.

THE PHOSPHOLIPIDS OF THE BRAIN, KIDNEYS AND HEART OF WHITE RATS IN EXPERIMENTAL HYPERTHYROIDISM

ARTHUR WEIL
Institute of Neurology, Northwestern University Medical School
CHICAGO, ILLINOIS

The profound changes which take place in the physiological function of the different organs of the body in experimental hyperthyroidism have repeatedly stimulated research, either microscopical or by chemical analysis, as to the underlying organic changes. Most of all, the changes in the activity of the central and peripheral nervous system, e.g. as revealed in the increased oxygen consumption or the changes in chronaxia of the cerebral cortex, invite an investigation of their chemical make-up.

Such an investigation would, naturally, be concentrated first on an analysis of the inorganic compounds and of the different lipids. Since the recent work of Sinclair (1), interest has again been focused on the rôle which the phospholipids, the important components of the central nervous system, might play under the conditions of such an artificially increased metabolism in experimental hyperthyroidism. Again the question arises, are they only intermediary products in the fat metabolism and is there a change in the amount and chemical composition if this metabolism is stimulated? Or, do they play an essential rôle in the physiological activity of the nervous system, and are they an integral part of its cytoplasm, acting as local catalysators? The present paper is concerned mainly with an investigation of the phospholipids of brain, kidneys and heart in the experimental hyperthyroidism as produced by feeding of thyroid gland powder to male white rats.

The literature on this subject is meagre. Only Parhon and Werner (2, 3) seem to have investigated the phosphorus content of the brain with more modern analytical methods in experimental hyperthyroidism and they were content to determine the total phosphorus with the help of the Derigès method in only small samples of fresh brains (90-150 mg.) without attempting to separate the different lipoid fractions. Furthermore, their short reports do not allow any conclusions as to the relationship of phosphorus values to sex and age. Their data seem to indicate the astonishing fact that subcutaneous injections of 1 mg. of thyroxin each day for 3 days into one rabbit increased the phosphorus of the brain by 33 per cent. Mazza (4) found in the brains of thyroid-fed guinea pigs a decrease in cholesterol and an increase in P. Most observers (5, 6) agree that in experimental and human hyperthyroidism the inorganic serum phosphorus is normal, while there is an increased elimination through the urine parallel with a decrease of the phosphates of the bones. Only Parhon and Cahane (7) report an increase of the inorganic P of the serum (20 per cent in 5 rabbits and 12 per cent in 1 dog) and a diminution of the

lipoid P of the serum in experimental hyperthyroidism (20 per cent in dogs and 13 per cent in rabbits).

The composition of the lipoids in the hearts of rabbits in experimental hyperthyroidism was studied by Fieschi (8). After feeding thyreoidin he found an increase in the amount of sterines, and of phosphorus in both the acetone and alcohol extracts. Despite an increase in weight, McEachern and Rake (9) found no qualitative changes in the hyperthyroid human heart from those found in so-called normal hearts of the same decade of life. An extensive review of the literature on the changes of heart, liver and kidneys in experimental hyperthyroidism is given by Simonds and Brandes (10). In their studies they found that, although the body lost weight both in experimental hyperthyroidism and in starvation, the heart increased in size and weight in experimental hyperthyroidism, while in starvation its weight was reduced in about the same proportion as the weight of the body as a whole.

TECHNIQUE

The following experiments were carried out in male white rats only. The rats had an average weight of 125 gm. when they were taken from our own

TABLE 1. FOURTEEN DAYS FEEDING OF THYROID POWDER. MALE RATS. MEAN VALUES OF WEIGHT OF BODY, BRAIN, KIDNEYS AND HEART.

Final Wt. (Theoretical) grams		Original wt. grams	Final wt. grams	Brain milligrams	Kidneys milligrams	Heart milligrams	No. of rats
145–175	Hyperthy.	155	140	1650	1430	710	22
	Controls	152	170	1640	1140	640	11
176–215	Hyperthy.	183	160	1695	1710	935	30
	Controls	187	203	1675	1425	750	20
216–235	Hyperthy.	220	202	1780	1920	1110	17
	Controls	233	245	1810	1575	915	10

stock or purchased from the stock of a single dealer. Two weeks previous to the beginning of the experiments they were separated and fed with the following diet: cracked yellow corn, 1200 gm.; casein, 320 gm.; powdered whole

TABLE 2. DIFFERENCES OF BODY WEIGHT AND WEIGHT OF ORGANS OF HYPERTHYROID AND CONTROL RATS. FIGURES INDICATE PER CENT DIFFERENCES FOR THE HYPERTHYROID RATS IN COMPARISON WITH THE CONTROLS. THEY ARE CALCULATED FROM TABLE 1.

Final weight (Theoretical) grams	Differences between original and final body weight		Differences between hyperthyroid rats and controls		
	Hyperthy.	Controls	Brain	Kidneys	Heart
145–175	−10	+12	+ .6	+25	+11
176–215	−13	+ 9	+1.2	+20	+25
216–235	− 8	+ 5	−1.7	+22	+21

milk, 400 gm.; alfalfa meal, 60 gm.; Na Cl, 10 gm.; and Ca CO_3, 10 gm. This same diet was also fed throughout the experiments. The rats were kept in pairs and each rat was allowed a daily ration of 10 gm. food per 100 gm. of body weight. Besides, for the production of hyperthyroidism, 100 mg. of

thyroid powder, U.S.P. (Armour and Co.) was added to the food of each rat daily. Controls were kept under the same conditions and were allowed the same amount of food minus thyroid.

At the end of 14 days the rats were killed by decapitation and bleeding. Brain, kidneys and heart were removed immediately, weighed and kept over night in 20 times their volume of acetone. Next, the organs were dried at 52° C. and the acetone extract was evaporated. Both were weighed and the sum gave the total dry substance for each organ. The difference between the fresh weight and that of the dry substance was calculated as water. The dried and pulverized organs were then extracted with absolute alcohol in a Soxhlet apparatus for 4 hours, dried and weighed. The difference between the weight before and after extraction was calculated as alcoholic extract. Phosphorus determinations were made for each organ separately in aliquotes of the acetone and alcoholic extracts and of the remainder of the organs left after extraction, using sulfuric acid and hydrogen peroxide for the wet ashing. The phosphorus in the acetone extract was determined in the combined extracts of 10 organs. The Fiske and Subarrow method was used for the phosphorus determination and the color intensity was determined with the aid of a photoelectric colorimeter.

Tables 1 to 6 contain the condensed results of the experiments. The figures represent mean values; the standard deviations have been calculated, but have been omitted for the sake of economy of space. They have been taken into consideration, of course, in drawing the general conclusions as to the basic differences between experimental and control groups. It is evident that only rats of the same original weight and sex can be compared under the same dietary and experimental conditions. The disregard of this rule has led to the many discrepancies among earlier publications. For practical purposes the weights in the final tables were arranged in 3 groups: 145-175 gm., 176-215 gm. and 216-235 gm., respectively. The figures under the heading of 'final weight (theoretical)' indicate that the final weights of the hyperthyroid rats had been corrected in such a way that they corresponded to the final weights of the controls of the same original weight at the beginning of the experiment.

COMMENT

The same manifold variety of reactions which the physician observes in human hyperthyroidism may also be seen in the rat experiments. The changes in body weight, for instance, may vary from an extreme of 30 per cent in reduction within 14 days to an actual increase up to 7 per cent of the original weight in isolated cases. Those rats which are most resistant to the loss of body weight may survive more than 3 weeks, while others under the same experimental conditions begin to die on the 12th day. The age of the animals seems to play an important rôle in this respect. Younger animals seem to lose relatively more weight than older animals, while the increase in weight of the controls follows the same rule (table 2). The decrease in body weight was proportional to the time of thyroid feeding, being 13 per cent after 14 days and 27 per cent after 21 days (table 5).

The changes in the weight of brain, kidneys and heart were more uniform than those of the body weight. Least marked were the differences between the brains of the hyperthyroid and control rats. Although these differences are within the possible experimental error, an analysis of figure 2, in which both curves of hyperthyroid and control rats run parallel, seems to indicate that in younger rats (150-190 gm.) the feeding of thyroid increased the normal

TABLE 3. WATER CONTENT; WEIGHT OF DIFFERENT LIPOID FRACTIONS; PHOSPHORUS CONTENT OF ACETONE AND ALCOHOLIC EXTRACTS AND THE REST OF EXTRACTION. THE LATTER FIGURES ARE CALCULATED IN PER MILLE, THE FIGURES FOR THE WEIGHT OF THE EXTRACTS IN PER CENT OF THE TOTAL DRY ORGAN. MEAN VALUES. THE NUMBER OF RATS IS THE SAME AS IN TABLE 1. FOURTEEN DAYS FEEDING WITH THYROID POWDER.

Final weight grams		H_2O	*Extract of* Acetone		Alcohol		*Phosphorus of* Acetone extract		Alcohol extract		Rest of extract		Total dry	
		%	mg.	%	mg.	%	mg.	‰	mg.	‰	mg.	‰	mg.	‰
						A. Brain								
145–175	Hyperth.	76.1	66	16.7	121	31.4	.73	1.8	3.06	7.8	2.04	5.1	5.83	14.7
	Controls	76.3	58	14.5	144	36.7	.68	1.9	3.07	7.8	1.92	4.9	5.67	14.6
176–215	Hyperth.	76.2	65	17.4	123	33.6	.79	2.1	3.05	7.2	2.15	4.9	5.99	14.2
	Controls	76.4	53	14.1	138	35.8	.72	1.8	3.03	7.7	1.91	5.1	5.66	14.6
216–235	Hyperth.	76.4	70	16.6	147	35.2	.85	2.1	3.08	7.2	2.09	4.8	6.02	14.1
	Controls	76.7	49	12.5	184	39.6	.65	1.6	3.16	7.8	1.83	4.6	5.64	14.0
						B. Kidneys								
145–175	Hyperth.	75.0	26	7.3	95	21.6	.51	1.3	1.63	4.1	2.54	6.5	4.68	11.9
	Controls	74.7	22	7.4	72	21.6	.40	1.5	1.28	3.9	2.17	6.6	3.85	12.0
176–215	Hyperth.	74.7	33	7.8	104	23.7	.54	1.3	1.76	4.5	3.02	6.2	5.32	12.0
	Controls	75.2	26	7.8	78	21.1	.44	1.1	1.46	4.3	2.33	6.3	4.43	11.7
216–235	Hyperth.	74.2	48	8.8	116	24.7	.70	1.7	2.05	4.0	3.37	6.1	6.12	11.8
	Controls	75.7	31	7.4	91	21.6	.46	1.3	1.80	4.3	2.56	6.1	4.82	11.7
						C. Heart								
145–175	Hyperth.	75.3	16	8.3	30	14.3	.24	1.3	.55	2.9	1.18	5.8	1.97	10.0
	Controls	76.3	11	7.3	26	16.4	.22	1.7	.44	2.9	.90	5.2	1.56	9.8
176–215	Hyperth.	75.2	19	8.2	34	14.3	.33	1.5	.70	3.1	1.35	5.5	2.38	10.0
	Controls	77.2	14	8.1	27	16.0	.23	1.6	.48	2.8	.86	4.4	1.57	9.8
216–235	Hyperth.	74.9	27	7.9	48	17.4	.45	1.7	.81	2.9	1.48	5.2	2.74	8.8
	Controls	77.5	18	9.1	32	15.7	.28	1.8	.56	2.8	.96	4.3	1.80	8.9

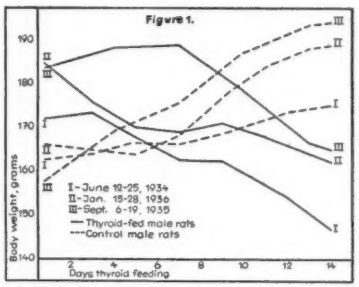
rate of growth of the brain. The differences in the weight of kidneys and heart were much more marked. Table 4 contains a summary of maximum, minimum and mean values together with the standard deviations of the mean. It seems that the relative increase in the weight of the heart was more pronounced in the older animals over 175 gm. weight than in the younger ones (table 2).

The increase in the weight of the kidneys paralleled the duration of the thyroid feeding, while the increase in the weight of the heart after 21 days

was less than after 14 days. Simonds and Brandes (10) made a similar observation in their thyroid-fed dogs. At the same time the differences in the chemical composition as compared with normal hearts were not as pronounced as in the 14-day experiments. These facts, perhaps, indicate that the great strain upon the physiological activities of the heart in the rat after 2 weeks leads to a breakdown of the normal mechanism of hypertrophy of the organ in response to increased activity. Table 5B gives a comparison between 2 groups of rats fed 14 and 21 days, respectively, with thyroid powder and a comparison of the chemical composition of the different hearts.

The changes in chemical composition of brain, kidneys and heart corresponded to the changes in their weight (fig. 3). Again, these changes were least pronounced in the brain.

Water content. In the 3 organs there was a definite tendency towards a decrease of water in experimental hyperthyroidism. Though the differences



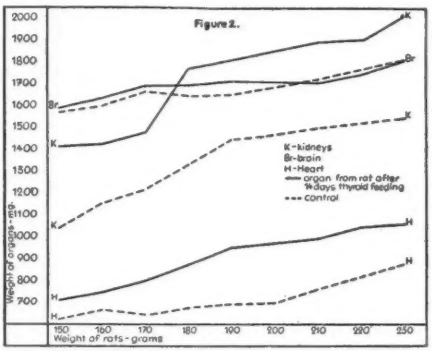


Fig. 1. Mean body weights of 20 male, hyperthyroid rats and 20 control males.
Fig. 2. Weights of brain, kidney and heart of experimental hyperthyroid and control male rats. Animals arranged in groups of 10 gm. difference in body wt. between each group. Abscissa = final wt. of 14-day controls which had the same initial weight as the hyperthyroid rats.

between experimentals and controls were so small that they fall well within the standard deviation, there was nevertheless a uniform tendency in the 3 different age groups (except kidney group 175-215) towards dehydration.

Acetone extract. The acetone extracts, containing neutral fats, esters of fatty acids, cholesterol and its esters, were increased throughout, more markedly in the older rats than in the younger ones. The phosporous of this extract (inorganic and small amounts of phospholipids) was barely changed in the brain but was increased definitely in the kidneys and more markedly in the heart.

Alcoholic extracts. In the brain there was a definite diminution in weight (16, 11 and 20 per cent) but practically no change in the phosphorus content of this fraction, indicating that the amount of phospholipids was not reduced, but that other substances (galactolipids?) had been used up. In kidneys and heart, both the weight of the alcoholic extracts and their phosphorus content were increased, in the heart relatively more than in the kidneys.

Remainder of extraction. There was a very minor increase in both weight and phosphorus content of the non-extractable portion of the brain containing the nucleoproteins and, perhaps, combinations of phospholipids with proteins. The differences between hyperthyroid rats and controls were more pronounced in the kidneys and still more in the heart in which both weight and phosphorus content increased in the same relation (45 and 54 per cent in the oldest group, respectively). This discrepancy between the marked increase in extracts and the rest of extraction in the hearts, as compared with the relatively much smaller increase in weight of the organ, finds its explanation in the decrease

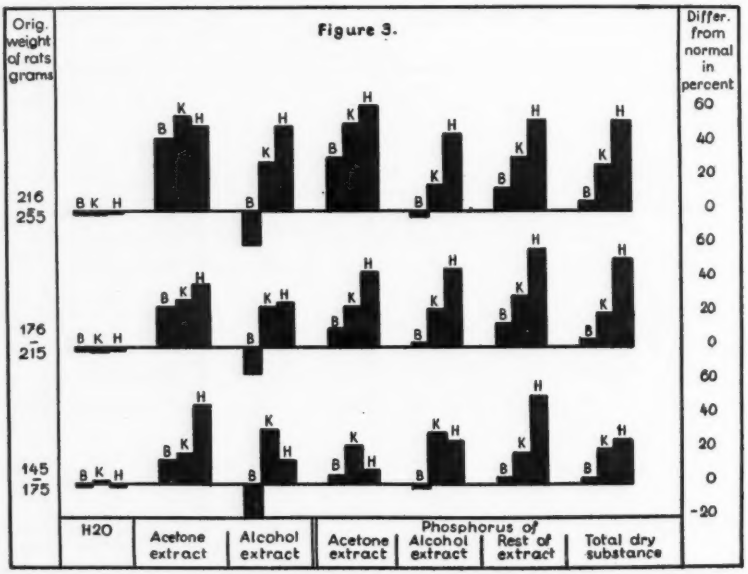


Fig. 3. Differences in chemical composition of brain (B), kidneys (K) and heart (H) between 14-day thyroid-fed and control male rats.

of water in the hearts of the hyperthyroid rats. Or, in other words, the functional hypertrophy of the heart is expressed in an increase of phospholipids and proteins of the heart muscle.

The total phosphorus was only mildly increased in the brain (3, 6 and 7 per cent, respectively, in the 3 groups) but by no means to such a degree as the experiments of former investigators (2, 3) might have indicated. On the other hand the increase of the total phosphorus of kidneys and brain paralleled their increase in weight (kidney, 48, 43 and 27 per cent; heart, 26, 51 and 52 per cent).

Qualitative changes. The question arises whether the increase in weight of kidneys and heart represents a physiological growth or whether there is a change in the chemical make-up of these organs in the hyperthyroid rats. The most exact but, at present, the most difficult and rather impossible method would be the isolation and chemical identification of the different components.

TABLE 4. WEIGHT OF KIDNEYS AND HEART. MAXIMA, MINIMA AND MEAN VALUES WITH STANDARD DEVIATION (S.D.) OF MEAN.

Body Wt. *grams*		Kidneys Max.	Min.	Mean	S.D.	Heart Max.	Min.	Mean	S.D.
		milligrams				*milligrams*			
145–175	Hyperth.	1740	1130	1430	±160	900	635	710	±115
	Controls	1320	940	1140	±130	760	555	640	±90
176–215	Hyperth.	1900	1365	1710	±175	1155	775	935	±110
	Controls	1590	1230	1425	±100	895	645	750	±60
216–235	Hyperth.	2160	1605	1920	±200	1305	925	1110	±100
	Controls	1675	1320	1575	±170	995	865	915	±60

However, there is a simpler though less accurate way to determine whether or not the phospholipids are changed qualitatively. It is the comparison of qualitative composition of the different extracts as expressed in percentage values. Table 3 indicates that there were only small differences in the percentage distribution of the total P despite large differences in the corresponding absolute values of hyperthyroid organs and controls. Furthermore, a calculation of the phosphorus content of the different fractions and of the relation of phospholipid P to total P indicates only minor differences between experiments and control (compare table 6).

The last column of table 6 indicates that there was no difference in the distribution of phosphorus between the alcoholic extracts and the non-extractable remainder in brain, kidneys and heart. Furthermore, the total extracts (acetone and alcohol combined) of the 3 organs had the same content of phosphorus both in hyperthyroid rats and controls. These results support the conclusion that the qualitative make-up of the phospholipids is not changed, although in hyperthyroidism their amount in kidneys and heart is increased proportionally to their increase in weight.

TABLE 5.

A. CHANGES IN THE WEIGHT OF BODY, BRAIN, KIDNEYS AND HEART OF MALE RATS FOLLOWING FEEDING OF THYROID POWDER. THIRTY RATS IN EACH GROUP. FINAL WEIGHT (THEORETICAL) 176–215 GRAMS.

	Original weight gm.	Final weight gm.	Loss %	Brain weight mg.	Brain increase %	Kidneys weight mg.	Kidneys increase %	Heart weight mg.	Heart increase %
14 days	183	160	−13	1695	+1.2	1710	+20	935	+25
21 days	172	125	−27	1551	−4.3	1738	+25	720	+13

B. CHEMICAL COMPOSITION OF HEART FOLLOWING 14 DAYS AND 21 DAYS FEEDING WITH THYROID POWDER. TWENTY MALE RATS IN EACH GROUP. FINAL WEIGHT (THEORETICAL) 175–215 GRAMS.

	H_2O %	*Extract of* Acetone mg.	%	Alcohol mg.	%	*Phosphorus of* Acetone mg.	‰	Alcohol mg.	‰	Remainder mg.	‰	Total mg.	‰
Hyperth. 14 days	75.2	19	8.2	34	14.3	.33	1.5	.70	3.1	1.35	5.5	2.38	10.0
Hyperth. 21 days	76.0	10	6.2	24	13.8	.22	1.3	.48	2.8	.90	5.3	1.60	9.4
Controls 21 days	77.8	9	6.7	20	14.3	.22	1.6	.39	2.8	.81	5.8	1.42	10.2

TABLE 6. PHOSPHORUS CONTENT OF THE DIFFERENT EXTRACTS AND RELATION OF PHOSPHOLIPID PHOSPHORUS TO TOTAL PHOSPHORUS. FINAL WEIGHT (THEORETICAL) OF MALE RATS 175–215 GRAMS. FOURTEEN DAYS FEEDING WITH THYROID.

		Acetone extract %P	Alcohol extract %P	Remainder of extract %P	Total extract %P	Alcohol. extr. P:Total Phosphorus
Brain	Hyperth.	1.12	2.48	1.07	1.53	1:1.91
	Controls	1.36	2.20	1.00	1.48	1:1.87
Kidneys	Hyperth.	1.63	1.69	1.04	1.24	1:3.02
	Controls	1.69	1.91	.91	1.22	1:3.04
Heart	Hyperth.	1.73	2.06	.74	1.01	1:3.41
	Controls	1.71	1.78	.67	.93	1:3.30

SUMMARY AND CONCLUSIONS

Feeding of thyroid powder to male rats was followed by a decrease of the body weight and an increased weight of kidneys and heart as compared with the organs of control rats of the same original weight. The loss of weight was relatively greater in younger animals than in older ones while the increase in heart weight was more pronounced in older rats.

There was a decrease in water, an increase of acetone soluble extracts in brain, kidneys and heart, an increase in alcoholic extracts in kidneys and heart and a decrease in the brain. The total phosphorus and the phosphorus of the different extracts of the 3 organs were increased proportionally to the increase in phospholipids and weight of the organ. Qualitatively the phospholipids were not changed, judging from a comparison of the percentage content of phosphorus and the constant relation between total and lipid-phosphorus in both hyperthyroid rats and controls.

The hyperthrophy of kidneys and heart in experimental hyperthyroidism of rats in response to an increased activity of these organs was reflected in an increase in weight and in a corresponding increase in the amount of their phospholipids.

REFERENCES

1. Sinclair, R. G.: J. Biol. Chem. **111:** 261. 1935.
2. Parhon, C. I., and G. Werner: Compt. rend. Soc. de biol. **104:** 443. 1930.
3. Parhon, C. I., and G. Werner: Compt. rend. Soc. de biol. **107:** 402. 1931.
4. Mazza, A.: Biochim. e terap. sper. **19:** 113. 1932.
5. György, P.: Handb. d. norm. u. pathol. Physiol. **16:** 1555. 1931.
6. Aub, J. C., W. Bauer, C. Heath and M. Ropes: J. Clin. Investigation. **7:** 97; 139. 1929.
7. Parhon, C. I., and M. Cahane: Compt. rend. Soc. de biol. **103:** 284. 1930.
8. Fieschi, A.: Ztschr. f. d. ges. exper. Med. **86:** 398. 1933.
9. McEachern, D., and G. Rake: Bull. Johns Hopkins Hosp. **48:** 273. 1931.
10. Simonds, J. P., and W. W. Brandes: Arch. Pathol. **9:** 445. 1930.

FAILURE OF THE REID HUNT ACETONITRILE REACTION AS A CLINICAL TEST FOR HYPERTHYROIDISM[1]

ROBERTO F. ESCAMILLA
From the Departments of Pharmacology and Medicine of the University of California Medical School
SAN FRANCISCO, CALIFORNIA

The acetonitrile reaction was first described by Reid Hunt in 1905. It is based on the reported fact that feeding thyroid to white mice causes an increased resistance to poisoning by acetonitrile. A rather extensive literature has accumulated on the subject, and since 1925 most of the publications have appeared in German periodicals. A clinical test for hyperthyroidism has been evolved which is said to be particularly valuable in early or mild forms of the disease.

Attempts by the author to duplicate some of the work gave results so contradictory that it was decided to make a more careful analysis, using newer methods and standards of bio-assay. Their application has cast serious doubt upon the validity of conclusions, interpretations and hypotheses reached by older, less exact procedures.

After his first papers in 1905 (1, 2), Hunt published a series of contributions on the subject. In a long article in 1923 (3) he summarized most of the work up to that time, stressing the fact that the reaction occurred only in white mice and that thyroid substance alone gave marked protection, also that resistance was increased on a limited diet and lowered by exercise and rapid growth. The young were less resistant than adults, and males less than females. There was a seasonal variation with more resistance in the winter and spring, and less in the summer and autumn. It was therefore necessary to have adult mice, of uniform stock, of the same age, same sex, and on a uniform diet for 7 to 14 days before the test was started. The minimal lethal dose of acetonitrile was said to be uniform to within 0.01 to 0.02 mg. per gm. of mouse. Under such conditions he stated that 1 mg. of desiccated thyroid could be detected. The protective effect seemed to vary with the iodine content of the thyroid (4, 5). It was also reported that the test had a quantitative value for large differences, that thyroid was more active than thyroxin and that thyroidectomy in mice lowered their resistance. Positive reactions were reported with blood taken from patients with hyperthyroidism and a few from patients with chronic nephritis. His last paper on the subject was in 1925 (6). In a recent personal communication Dr. Hunt stated that he thought the reaction useful for detecting the presence of thyroid in obesity remedies but that he now had little confidence in it as a clinical test for hyperthyroidism.

Ghedini (7) in 1911 reported confirmatory results using blood from patients with Basedow's disease and concluded that the test showed promise. His paper started a wave of interest and investigation in Germany. Von Bergmann (8, 9)

[1] Assisted in part by the Christine Breon Fund for Medical Research.

described the condition of 'vegetative stigmata' and noted that this group occasionally showed positive Reid Hunt reactions even when their metabolisms were normal, suggesting a probable relationship to hyperthyroidism. Goldner (10) discussed the manner of performing the test and reported that the subcutaneous injection of 0.5 cc. of blood serum on 3 successive days worked just as well as feeding the mice whole blood, as had been done up to that time. Another paper by Goldner (11) is frequently cited as a reference for the method of performing the test. He stressed again the necessity for constant temperature and diet, and reported oats and water as the diet to be used during the experiment. Oehme and Paal (12) have published the most recent lengthy monograph on this test, with a complete survey of the literature. They reported the results of 813 tests, obtaining positive results in patients with Basedow's disease and occasional positives in vegetative stigmata, asthma, Parkinson's disease, tachycardia and uremia. They believed that insulin might destroy the effect in diabetes and also noted that "adrenalin irregularly protected against the resistance-destroying effect of insulin." Anterior pituitary extracts usually gave slightly positive results. The tests for urine and blood were compared and found to be more frequently positive with urine. They concluded that the test was probably due to increased thyreotropic hormone from the anterior pituitary in the bloodstream and urine, occasionally in amounts not sufficient to cause hyperactivation of the thyroid.

Occasional unfavorable comments have appeared in the literature. Carlson and Woelfel (13) in 1910 noted individual variations in groups of mice so that a sharp minimal lethal dose for acetonitrile was difficult to determine. Similar difficulty has since been reported by others (14, 15, 16, 17). Trendelenberg (18) reported that feeding blood from thyroidectomized cats, from mice fed with thyroid and from patients with Graves' disease all had the same effect. Neuweiber (19) reported only negative reactions with pregnancy serum, progynon, and prolan. In 1935, Wokes (20) reported his experience with the test, using the modern methods of bio-assay. He found some protective effect at room-temperature with thyroid fed by a stomach-tube, but the toxicity curve was too flat to make the test practicable. He concluded that it had little value clinically and that, inasmuch as the protective effect was diminished at a high temperature where the curve was steeper it would be of no value in that range. There was absolutely no quantitative effect.

PROCEDURE

The procedure for the clinical test as outlined by Oehme and Paal (12) is as follows. Male white mice of adult age are placed in a room with fairly constant temperature and kept on an unvarying diet of rolled oats and water for at least 2 weeks. Then the minimal lethal dose of acetonitrile for this particular colony is determined, following which Reid Hunt tests are performed by taking blood from the patient, separating the serum, and injecting 0.5 cc. of the serum subcutaneously in 5 mice on each of 3 successive days. On the 4th day the mice are poisoned with graded doses of acetonitrile subcutaneously, using increasing fractions of the minimal lethal dose, such as 3/3, 4/3, 5/3, 6/3 and 7/3. Survival of 1 mouse indicates a positive test. If the only survival is the mouse which receives 3/3, or the actual minimal lethal dose, the test is considered weakly positive. The greater the number of survivals, the more strongly positive the test.

It was decided to try this first but, after the mice had been prepared with

the diet of oats and water, efforts to find the minimal lethal dose by injecting graded doses of acetonitrile were unsuccessful. Table 1 shows the total results of this effort to find the minimal lethal dose by this method. Examination of this table reveals that 75 per cent of mice injected with 0.3 mg. per gm. died, while only 44 per cent of those injected with the larger dose of 0.9 mg. per gm. died. This was at surprising variance with Hunt's

TABLE 1. TOTAL RESULTS OF MINIMAL LETHAL DOSE TESTS IN MICE ON DIET OF OATS AND WATER.

Dose (mg. per gm. mouse)	No. Living	No. Dead	
0.1	1	1	
0.2	2	2	
0.3	1	3	
0.4	0	1	
0.5	3	7	Total
0.6	2	7	69
0.7	2	8	mice
0.8	4	7	
0.9	5	4	
1.0	3	6	

report (2) that the minimal lethal dose of acetonitrile in mice was remarkably uniform and could be ascertained sharply to within 0.01 to 0.02 mg. per gm. of mouse. Furthermore, if the minimal lethal dose was so uncertain for this group of 69 mice it would seem that the numerous positives reported, if 1 mouse of 5 recovered after receiving 4/3 of the m.l.d., should rather be recognized as normal variations in reaction to the poison. Incidentally, in all the literature reporting such tests, there is practically no comment about the manner in which the lethal dose was determined or the number of mice used.

DIET

Since occasional spontaneous deaths in the colonies of mice were proved by post-mortem examination to be due to pneumonia or pulmonary congestion, it was decided that the diet might be partly at fault. Prolonged feeding with oats and water only, causing an obvious deficiency in calcium and vitamins A and D, might well make them more susceptible to infections. The susceptibility of the mouse to pneumococcus infections is a well-known fact. Therefore, it was decided to feed the mice the diet used by the Hooper Foundation for Medical Research at the University of California. The composition is as follows: rolled oats, 240 parts; skimmed milk powder, 30 parts; cod-liver oil, 8 parts; salt, 1 part. The various ingredients are first thoroughly mixed by hand and then run through a food-grinder so that the mixture is as nearly homogeneous as possible, and the mice have a uniform intake of the various components. From this point on, autopsies were performed on most of the mice dying either during the course of the experiment or spontaneously and notes made of gross pathological changes.

METHODS OF BIO-ASSAY

Comparisons were then made using the methods of biological assay ad-

vocated by Trevan (21) and Burn (22). With this method the factor of animal variation is considered, and the percentage of reaction at the same dose is shown to give a much more accurate determination of lethal dose than the diminishing dose-scales used above. If this percentage is determined at several different levels, a toxicity curve can be constructed which will be characteristic of the reaction of that species to that drug. Enough animals should be used at each dose so that the percentage of standard deviation will

TABLE 2. RESULTS OF REID HUNT ACETONITRILE TESTS.

Patient and Sex	Age	Pathology of Thyroid gland	Duration of Symptoms	Basal Metabolic Rate Per Cent	Dose of Acetonitril Used in Poisoning Mg./gm.	Results Dead—Living	Test
1. Z.M. F	54	Toxic hyperplasia	Previous operation and X-ray therapy	+14.3	0.25	5—0	Negative
2. V.R. F	26	Toxic hyperplasia	8 months	+52.7	0.25	5—0	Negative
3. A.B. M	54	Toxic hyperplasia	3 months	+64.0	0.2	5—0	Negative
4. L.D. F	37	Toxic hyperplasia and thyroiditis	3 years	+86.9	0.2	5—0	Negative
5. E.P. F	53	Toxic hyperplasia (also diabetic)	19 months; previous X-ray therapy	+55.1	0.2	5—0	Negative
6. K.M. F	19	Toxic hyperplasia	7 months; more toxic later	+13.4 (later +63.2)	0.2	3—2	Negative
7. E.A. F	29	Diagnosis—anxiety neurosis	? mild toxic symptoms 5 years before	+ 2.9	0.2	1—4	Positive
8. A.L. F	52	Multiple adenomata	Previous operation; present symptoms 3 years	+10	0.2	4—1	Negative
9. A.C. M	34	Toxic hyperplasia	3 months	+64.3	0.2	6 mice used; 1 died during serum injections; of remainder 4—1	Negative
10. M.L. F	43	Toxic hyperplasia	1 year; nearing crisis on entry	+70	0.2	4 died during serum injections 1—0	?
11. J.D. F	21	Diagnosis—early schizophrenic	Nervous for 2 years	−18	0.2	3—2	Negative
12. Normal M	29			+ 0.2	0.25	5—0	Negative

not be high. The formula for determination of percentage of standard deviation is given and explained in Burn's paper. Other important points emphasized are that submaximal doses should be used, that reliance on differences is more important than on equality, and that apparently discrepant results must always be included.

For this experiment it was therefore necessary to construct such a toxicity curve showing the reaction of this group of white mice to acetonitrile. Then, taking a point on the curve where the percentage of deaths was high, 5 mice could be treated with blood-serum from the patient by subcutaneous injection of 0.5 cc. on 3 successive days, and on the 4th day all injected with the same dose of acetonitrile. Any increase in the percentage of survivals which exceeded the standard deviation for that dose would therefore indicate some protection.

A new colony of male mice was obtained and placed on a complete homogeneous diet as outlined above. After 2 weeks, they were tested with the following results.

Dose (mg. per gm. mouse)	*Living*	*Dead*
0.05	2	0
0.075	1	1
0.1	28	8
0.2	9	18
0.25	1	4
0.3	2	22
0.4	0	2
0.5	0	12
0.6	0	3
0.7	0	1
0.8	0	1
0.9	0	1
1.0	0	12

The only points with enough mice to be included in the curve were at doses of 0.1, 0.2, 0.3, 0.5 and 1.0 mg. per gm.

It is of further interest to note that for the 85 deaths occurring in this series there were 75 autopsies performed. Of these, 13 showed pneumonia, 8 showed pulmonary congestion, all had enlarged spleens and 1 had a granular-appearing liver. All of these mice appeared healthy at the time of the injections. It was therefore decided to revise the totals so that the series did not include the mice with evidences of pulmonary infection. Therefore:

At a dose of 0.1 mg. per gm.—28 lived, 4 died.
At a dose of 0.2 mg. per gm.— 9 lived, 12 died.
At a dose of 0.3 mg. per gm.— 2 lived, 17 died.
At a dose of 0.5 mg. per gm.— 0 lived, 11 died.
At a dose of 1.0 mg. per gm.— 0 lived, 11 died.

This factor of illness among the laboratory animals is not apparent before they are used, and should certainly be considered as affecting toxicity tests. There should be more reports of autopsies on animals dying as a result of experimental procedures in the laboratory.

A toxicity curve was then constructed (fig. 1), and 2 doses were used in several Reid Hunt tests. The first was 0.25 mg. per gm. (where approximately 80 per cent should die), and the second 0.2 mg. per gm. (where approximately 57 per cent should die). Five mice were used for each test, being injected with 0.5 cc. of serum from the patient on 3 successive days. On the 4th day they were poisoned, all with the same dose of acetonitrile. Twelve tests were performed on various patients. All the mice were autopsied after death and there was a rather high incidence of pulmonary infection. The results of these tests are given in table 2.

Only 1 of the 12 tests can be called positive and that was in a patient with a basal metabolism of only +2.9 per cent. On the other hand, obviously toxic patients, with metabolisms of +64.3 and +86.9 per cent, had negative tests, and in fact it was remarked during the course of the experimental work that the mice receiving serum from patients that were very toxic, appeared ill

on the second and third days, and occasionally died beforehand or succumbed very quickly to the injections of acetonitrile. From this, the reasonable conclusion is that the test has no value clinically in detecting thyroid hyperfunction, either as the hormone from the thyroid itself or as the pituitary thyreotropic hormone in the blood-stream, and that previously reported positive clinical results are most probably due to the natural variation in the resistance of the mice to acetonitrile. It is therefore easy to understand why so many contradictory findings have been reported.

No effort has been made here to check the work of Hunt in which thyroid

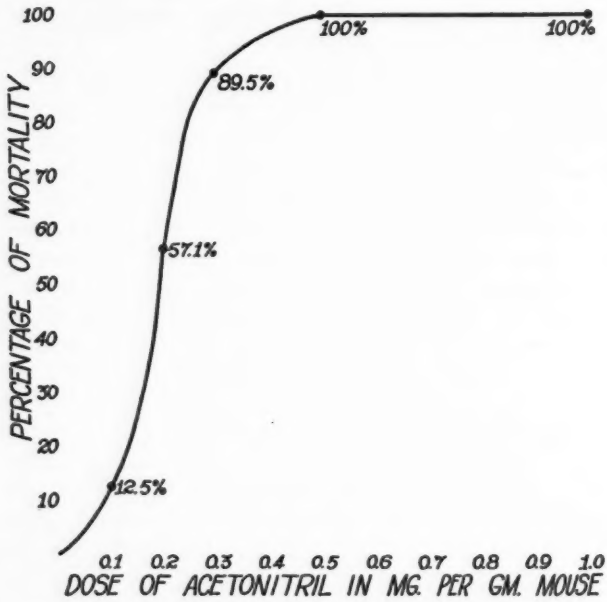


Fig. 1. Characteristic toxicity curve for acetonitrile test with mice. Diseased mice were not included.

in relatively large doses was fed to mice, but the recent report by Wokes, (20) using the methods of biological assay suggested by Trevan and Burn and followed here, showed that there was some definite protection of white mice against acetonitrile by thyroid. However, this protective effect seemed to disappear at higher temperatures, and at room-temperature the toxicity curve was so flat that it was difficult to apply the acetonitrile test.

Therefore, the protective effect, when analyzed by these more critical methods of biological assay, is not as great as had been formerly supposed and, in view of the results reported in this paper, the test is not applicable clinically. This work seems a good example of the increased accuracy of results in bio-assay made possible by the methods of Trevan (21) and Burn (22). The importance of their wider application is obvious.

SUMMARY

The history of the Reid Hunt acetonitrile test is briefly surveyed. Efforts to repeat some of the work with a view to clinical application were unsuccessful because of the apparent impossibility of finding a sharp minimal lethal dose for acetonitrile in white mice.

Using the method of Trevan and Burn, a toxicity curve was constructed for the effect of acetonitrile on white mice, and 12 Reid Hunt tests then attempted. Eleven were negative, and one positive. The patient whose blood caused a positive test had a basal metabolic rate of +2.9 per cent, while among the negatives were very toxic patients with rates between +64.3 and +86.9 per cent.

Other factors that might influence toxicity tests, such as inadequate diet and intercurrent infection in the laboratory animals, were corrected by giving a complete diet, and performing post-mortem examinations—ruling out animals that showed gross evidence of pulmonary infections.

As a result of this work, the Reid Hunt acetonitrile reaction is not recommended as an aid in the diagnosis of thyroid function.

Note: Since the completion of this paper an article by Wiesbader (23) has appeared reporting positive Reid Hunt reactions using human pregnancy serum, and also after injections of Follutein (Squibb), and of anterior pituitary thyrotropic hormone. It is interesting to note that he used mice prepared on the standard (but deficient) diet of oats and water, and that increasing-dose scales were used in performing the tests. Furthermore, an arbitrary figure was given for the lethal dose with no details concerning its manner of determination.

REFERENCES

1. Hunt, R.: J. Biol. Chem. **1:** 33. 1905.
2. Hunt, R.: Proc. Soc. Exper. Biol. & Med. **3:** 15. 1905.
3. Hunt, R.: Am. J. Physiol. **63:** 257. 1923.
4. Hunt, R., and A. Seidell: J.A.M.A. **51:** 1385. 1908.
5. Hunt, R., and A. Seidell: Hygienic Laboratory Bulletin No. 47, 1909.
6. Hunt, R.: Pub. Health Rep. **40:** 1461. 1925.
7. Ghedini, G.: Wien. klin. Wchnschr. **24:** 736. 1911.
8. von Bergmann, G.: Med. Klin. **24:** 844. 1928.
9. von Bergmann, G.: Ztschr. f. klin. Med. **108:** 90. 1928.
10. Goldner, M.: Ztschr. f. klin. Med. **108:** 110. 1928.
11. Goldner, M.: Ztschr. f. klin. Med. **114:** 456. 1930.
12. Ohme, C., and H. Paal: Ergebn. d. inn. Med. u. Kinderh. **44:** 214. 1932.
13. Carlson, A. J., and A. Woelfel: Am. J. Physiol. **26:** 32. 1910.
14. Port, F.: Biochem. Ztschr. **51:** 224. 1913.
15. Gaddum, J. H., and M. Hetherington: Quart. J. Pharm. & Pharmacol. **4:** 183. 1931.
16. Eisler, B., H. Hennig and A. Schittenhelm: Ztschr. f. d. ges. exper. Med. **86:** 331. 1933.
17. Montgomery, E. H.: Yale J. Biol. & Med. **6:** 101. 1933.
18. Trendelenburg, P.: Biochem. Ztschr. **29:** 396. 1910.
19. Neuweiber, W.: Zentralbl. f. Gynäk. **56:** 2936. 1932.
20. Wokes, F.: Quart. J. Pharm. and Pharmacol. **8:** 54. 1935.
21. Trevan, J. W.: Proc. Roy. Soc., London, s.B. **101:** 483. 1927.
22. Burn, J. H.: Physiol. Reviews **10:** 146. 1930.
23. Wiesbader, H.: Endocrinology **20:** 100. 1936.

CLINICAL AND LABORATORY NOTES

REPORT OF 1600 ASCHHEIM-ZONDEK TESTS (FRIEDMAN MODIFICATION)*

MORRIS FERESTEN

The object of this report is to describe a simpler technic for the Friedman modification of the Aschheim-Zondek test, which has proved as accurate as more complicated procedures.[1, 2] The results of 1600 tests indicate its practicability. Methods to avoid certain pitfalls encountered in the earlier tests are also described.

The Friedman modification is based on the fact that corpora lutea and corpora hemorrhagica occur in the rabbit's ovaries only after copulation, or artificially from the injection of a pituitary-like gonadotropic substance present in the urine of pregnant women.

TECHNIC. *Rabbits.* The rabbits used were ordinary mature, domestic females, weighing 5 to 7 lb. or more. Ovaries of larger rabbits were found to be more sensitive to the gonadotropic hormone than those of smaller immature animals. Also, the larger animals were better able to tolerate a single intravenous injection of 25 cc. of urine, as advocated in this technic.

When possible, rabbits were isolated in individual cages for 1 month, their period of gestation. This prevents the use of gravid rabbits as well as those with pseudopregnancy. When necessary, however, rabbits separated for a shorter time were used with no untoward results. After a week, the corpora lutea of pregnancy and pseudopregnancy could not be confused with the fresh corpora lutea or the large bulging corpora hemorrhagica produced by the gonadotropic hormone of pregnant women.

Urine. Patients are not allowed oxytocics for 24 hours before the specimens are obtained. Pus and large amounts of bacteria in the urine were also found harmful. These factors caused the death of 19 rabbits. However, the same specimens, when detoxicated by the method of Zondek,[3] were injected with no untoward results. This procedure consists of shaking the urine several times with ether, which is then decanted. Liquids are restricted after 8 P.M., so that the voided specimens obtained the next morning will be more concentrated. Cleanliness, but not asepsis, is necessary for obtaining, storing and injecting the specimens.

If the specimens are not injected soon after voided they should be kept in a refrigerator. They are warmed before use but not overheated, as prolonged heat causes a loss of potency. The urine is filtered before injection to remove any precipitate. In this series, specimens have been kept as long as 4 days on ice, and in several instances have been sent through the mail with no loss in potency.

Method of Injection. The rabbit is placed in a restraining box, with its head protruding through an opening. This allows the injection to be easily done by one

* From the Laboratory of the Park East Hospital, New York, N.Y.

[1] Aschheim, S., and B. Zondek: Klin. Wchnschr. 7: 1404. 1928.
[2] Friedman, H. M., and M. E. Lapham: Am. J. Obst. & Gynec. 21: 405. 1931.
[3] Zondek, B.: Klin. Wchnschr. 9: 964. 1930.

person. The marginal veins are preferable and are made prominent by rubbing them with xylol or ether. A 22 ga., 1.5 in. needle is inserted into the vein and is held firmly with the ear between the thumb and forefinger of the left hand, so that it will not be dislodged when the rabbit moves. Instead of repeated small amounts, 25 cc. of urine is slowly injected at one time over a period of 5 to 10 minutes. This quantity will be well tolerated if large rabbits are used and the urine is not toxic. When the needle is removed, pressure is applied to the site of injection until bleeding ceases. Rabbits are conveniently identified by writing the patient's name inside of their ears with indelible ink or dye.

Interpretation of the Ovarian Reactions. The rabbits are autopsied 48 hours after injection. An intravenous injection of 20 cc. of air kills them instantly. If the ovaries contain only clear, unruptured follicles, the reaction is negative. If the ovaries contain fresh corpora lutea or large bulging corpora hemorrhagica, the reaction is positive. It was found more reliable to depend essentially upon the presence of corpora hemorrhagica for the diagnosis of pregnancy. They appear as large, bulging elevations on the surface of the ovary, contain fresh blood clots and are clearly visible to the naked eye. Lenses and microscopic sections are unnecessary. Whenever there is doubt as to the interpretation of results, it is always best to repeat the test. This was necessary 8 times in the present series.

Usually, 1 rabbit was used for each test. However, when time was the important factor, as for the diagnosis of extra-uterine pregnancy, 2 rabbits were injected. One rabbit was autopsied 36 hours after injection. If the results were positive the test was considered conclusive, if negative the final report was deferred for 12 hours when the other rabbit's ovaries were examined. Whenever the results were negative and the clinical course still suggested pregnancy, the tests were repeated within a week.

RESULTS. There were 659 positive and 941 negative reactions in the tests performed for the early diagnosis of pregnancy. One test was positive 2 days before the patient's expected menses. However, inasmuch as most of the false negative reports occurred when the test was made too early, it should not be performed less than a week after the patient's expected period.

False Reports. The percentage of error for the Friedman modification is much higher than is apparent from reported statistics. The closest follow-up can hardly discover all errors. Some patients, whose reports are positive, drift to abortionists who have no scruples about curetting a nongravid uterus. Others, whose test results are negative, change physicians when their menses do not occur. Many positive test reactions in this series were from patients who had received negative reports elsewhere. One negative result was from a patient whose urine had been reported positive by a reliable laboratory 2 days before. Several days later the patient had a normal menstrual period. However, as far as could be determined from a questionnaire of the doctors for whom these 1600 tests were performed, there were 15 false reports (.94 per cent).

In 13 cases in which a negative-result test was repeated after a week because the clinical findings still suggested pregnancy, the second test gave a positive reaction. It is hardly fair to call 6 of these false negative reports errors, as they were from patients who were less than a week overdue, the time interval being too short for the production of sufficient gonadotropic hormone. In the remaining 7 cases, the false negative reactions were probably due to animals being autopsied before 48 hours had elapsed, insensitivity of the rabbit's ovaries to the gonadotropic substance, or to a low urinary excretion of the hormone.

There were 2 false positive reports. In retrospect it is evident that both of these errors could have been avoided. In one case of menopause with increased prolan production, the rabbit's ovaries contained small follicular hemorrhages but not the characteristic large bulging corpora hemorrhagica with blood clots. In the other case, small ovarian follicles with hemorrhagic degeneration were misinterpreted as a positive result.

There probably would have been fewer errors if 2 rabbits had been used for each test. However, it hardly seems worthwhile to have sacrificed 1600 additional rabbits to produce a fractional increase in accuracy. Repetition of the 13 false negative-result tests gave corrected positive reports with little inconvenience to the patients. If attention is paid to details of technic and the test repeated whenever there is any question as to interpretation, the percentage of error will be brought to a satisfactory minimum, with the use of 1 rabbit.

Tubal Pregnancy. Urines from 7 cases of tubal pregnancy, with viable chorionic villi, gave positive reactions. Two rabbits were injected for each test. One rabbit was autopsied at the end of 36 hours and if the results were negative the other was examined 12 hours later, when a final interpretation was made. All of the animals autopsied at the end of 36 hours gave positive reactions.

Gravid Rabbits. Experimentally, 28 gravid rabbits were injected with urines to be tested for pregnancy. In each case the results, checked with those of nongravid animals, were found correct. Old corpora lutea, but no fresh corpora hemorrhagica, were present in the 13 gravid rabbits injected with urine from the patients who were not pregnant. In 15 cases large bulging corpora hemorrhagica gave conclusive evidence that the patients were pregnant. Rabbits which had recently delivered a litter were found particularly satisfactory for the test.

Results after Curettage. In 2 cases an opportunity was afforded to examine the urine for gonadotropic hormone daily after the products of conception were removed by curettage. In 1 case the pregnancy test reaction remained positive for 3 days and in the other for 6 days following the curettage.

SUMMARY. A simplified procedure for the Friedman modification of the Aschheim-Zondek test is described which requires: *A)* the use of ordinary mature, domestic, female rabbits, weighing 5 to 7 lb. or more, which have been isolated in individual cages for 31 days; *B)* a single intravenous injection of 25 cc. of filtered morning urine; *C)* an interval of 48 hours for the interpretation of the ovarian reactions; *D)* the presence of large bulging corpora hemorrhagica for a positive diagnosis.

The results of the 1600 tests performed for the early diagnosis of pregnancy were positive in 659 and negative in 941 instances. There were 13 false negative reports, 6 of which were from patients less than a week overdue. There were 2 false positive reports, which in retrospect appear to have been errors of interpretation. The total percentage of error was .94 per cent. Seven cases of tubal pregnancy, with viable chorionic villi, had positive tests.

Experimentally, 28 gravid rabbits were injected with urines to be tested for pregnancy. In each case the diagnosis proved correct as checked with the results of nongravid rabbits.

In 1 case the test for pregnancy remained positive for 3 days, and in another for 6 days after the removal of the products of conception by curettage.

1141 Park Ave.,
New York, N.Y.

THE PRESSOR EFFECT OF PROLONGED ADMINISTRATION OF GLYCERIN EXTRACT OF ADRENAL CORTEX*

R. G. HOSKINS AND J. H. FIERMAN

In previous publications[1] attention has been called to the fact that glycerin extract of adrenal cortex administered by mouth causes an increase in the systolic and diastolic blood pressure. In our experience, most schizophrenic patients and a considerable proportion of non-psychotic control subjects have shown this reaction. In the work previously reported, the dosage used was commonly 2 grams (fresh gland equivalent) 3 times daily and the period of treatment 3 weeks. This paper reports the results of varying the dosage and prolonging the periods of medication.†

Seven known 'reactors'—all subjects of the schizophrenic psychosis—were started at a dosage of 0.65 gm., t.i.d. This was continued for 40 days, then increased to 1.30 gm. The dosage at the end of each 40-day period was augmented by 0.65 gm. The final dosage employed ranged in the various cases from 1.95 to 3.25 gm., t.i.d. The time interval varied from 120 to 200 days.

The average pressure for the 7 patients prior to the beginning of medication was 108/67 mm. Hg. At the end of this first period, it was 118/74.

During the second and third periods, the dosage being 1.30 and 1.95 gm. t.i.d., respectively, the pressures continued to increase, the averages being 127/81 and 130/86. These periods were marked by a characteristic irregularity. Although the total trend was upward, in each of the individual cases during either the second or third period there was a plateauing for about a month or even a slight falling off of the pressure. In 1 instance, this falling off was postponed until the fourth period when a dosage of 2.60 gm. was being used. This plateauing is reflected in the relative slightness of the increase in the third period. The cause of the phenomenon could not be determined but the consistency of its occurrence indicates that it was other than a random variation. The possibility has to be considered that it may have been due to a seasonable decline in the content of the pressor principle of the glands used or a seasonal variation in the cardio-vascular system. At any rate, it took place in the interval during June and July.

After the third period, the course of the experiments became irregular. The last 2 patients of the series were discontinued at this level. Of the remaining 5, 3 were carried through a fourth period at 2.60 gm. t.i.d. Two others, after 4 days of treatment at the 2.60 gm. level, were showing such marked pressor effects that it seemed to be the part of good clinical judgment to reduce the dosage—in the one case to 1.30 gm. and in the other to 1.95 gm. Nevertheless the pressure continued to rise for 15 and 20 days, respectively, reaching the levels of 178/104 and 170/112 mm. In the 3 patients carried through the 2.60 period, 1 showed the secondary falling off previously mentioned, reaching 120/85, and the other 2 increased from

* From the Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital, Worcester, Massachusetts.

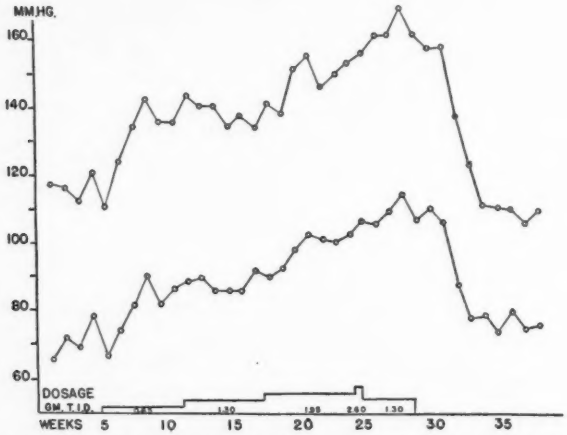
† We are further indebted to Schieffelin & Co., of New York for the large quantities of the extract that were needed for this test.

[1] Hoskins, R. G. and H. Freeman: Endocrinology **17**: 29. 1933.
Freeman, H., F. E. Linder and R. G. Hoskins: Endocrinology **17**: 677. 1933.
Freeman, H., and R. G. Hoskins: Endocrinology **18**: 576. 1934.

115/75 and 120/85 to 130/95 and 145/90 mm., respectively. Only 1 patient was continued through a 3.25 gm. period. In his case, the pressure increased during that period from its secondary depression of 120/85 to 130/90 mm.

In the cases previously reported in which smaller dosage and shorter periods of time were employed, it had been noted that after discontinuance of the treatment, the pressor effect gradually fell off, the original level being regained in from 2 to 3 weeks. In the present series with a higher terminal pressure about 30 days was required for the complete subsidence of the effect.

One of the purposes of the experiment was to determine whether an ultimate plateau would be reached beyond which increase of dosage would be ineffective. In the 2 instances mentioned what seemed to be the practical limit of dosage was reached with the systolic pressure going on individual days above 180 mm. Having



Systolic and diastolic blood pressure of a schizophrenic patient who received glycerin extract of adrenal cortex by mouth. Each circle represents the average of 3 determinations with one or two days intervening between each.

passed through the intermediate plateau, the pressures in the other cases were all still rising when the treatment was discontinued. The data seem to indicate that the extract is capable of increasing the pressure to any desired extent at least up to 200 mm.

As is well known, when adrenin is administered intravenously in minimal effective dosage, a fall of blood pressure is often seen. With increased dosage, this changes to a rise. We were interested to see whether a similar diphasic response to the glycerin extract could be demonstrated. At the minimal dosage a pressor effect occurred in 6 cases and no appreciable effect in the seventh. The fact that 1 of the patients failed to react and that the reaction was notably gradual in its appearance in the others suggests that the dosage of 0.65 gm. t.i.d. was at the threshold of effectiveness. Had the material a diphasic effect there might have been expected an initial drop in pressure before the patient came fully under the influence of it.

The accompanying graph serves to exemplify the general course of the reactions. The average pressure each week is plotted through a period of 37 weeks during which interval the dosage was increased from 0.65 to 2.60 gm. t.i.d., reduced to 1.30 gm., then discontinued.

Our primary aim in making the test was to determine whether the product had

any significant therapeutic effect on the psychosis. Despite the prolonged pressor reactions that were secured, no significant improvement in the mental symptoms could be detected.

Summary. Glycerin extract of adrenal cortex was administered orally to 7 schizophrenic patients in dosage gradually increased at 40-day intervals. The initial dosage in all cases was 0.65 gm. (fresh gland equivalent), 3 times daily. The terminal dosages ranged from 1.95 to 3.25 gm., t.i.d. No reversal of the pressor influence with small dosage could be detected. The threshold of responsivity was approximately at the minimal dosage used. In general the systolic and diastolic blood pressures gradually increased throughout the course of the treatment but a plateauing or slight reversal of the pressor trend was seen in all cases at a dosage of about 2 gm. t.i.d. In 2 instances the test was discontinued because of the high pressure reached (circa 170 mm.).

DELAYED AND INCOMPLETE UNION OF THE EPIPHYSES OF THE LONG BONES*

E. E. Beard

A case is presented because it shows the very unusual condition of incomplete epiphyseal union at the age of 44 years.

A. S., white male, age given as 31 years, was first admitted to the Medical Service of Cleveland City Hospital in February, 1929, with the complaint of pain in the chest. He was discharged with the diagnosis of hypopituitarism. He had then a mild anemia, an increased glucose tolerance, a basal metabolic rate of —11 per cent, and his sella turcica was of normal size and configuration by x-ray. He was readmitted to the same hospital in January, 1936. Records of the Probate Court and the Bureau of Vital Statistics established his age as 44 years at the time of examination.

The patient's intelligence was sub-normal and his story certainly unreliable. He had once been an inmate of an institution for the feeble-minded. His appearance was typical of eunuchoidism. His facial expression, the shortness of his stature and his high-pitched voice gave him somewhat the appearance of youth, which was belied, however, by the lined, atrophic appearance of his skin. The hair of the head was fairly thick, black and of a fine texture. On the face there was only a very little down (the patient said that he had never shaved), and there was no hair on the chest or abdomen, none in the axillae or about the anus and only a few hairs in the pubic region. The disposition of subcutaneous fat was typical of eunuchoidism. The breasts were those of an adolescent girl and the trochanteric region was padded. The penis was very small; the testes were not larger than 1 cm. in any diameter; the prostate could be felt only as a thickening of the wall of the rectum. Body measurements were as follows: total height, 64.0 in.; span, 68.2 in.; upper height, 27.5 in; lower height, 36.5 in.; circ. of head, 21.8 in.; circ. of chest, 33.8 in.; circ. of abdomen, 34.0 in.; circ. at trochanters, 38.5 in.; weight, 140 lb.

Laboratory investigation. Urine specimens were normal on routine examinations. Urinary output for 24 hours on unlimited intake reached 2200 cc. on one occasion but usually ranged between 1200 and 1500 cc. A 48-hour urine specimen assayed for androtin showed an average comb growth in each of 2 caponized birds

* From the Endocrine Clinic, Cleveland City Hospital and the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio.

of 0.5 mm., a completely negative result.† Urine, 186 cc., concentrated with tannic acid, failed to give a positive test for prolan.‡ Two B.M.R. determinations made at an interval of 1 week were —41 per cent and —35 per cent, and the simultaneous blood cholesterol values were, respectively, 220 and 190 mg. per cent. Glucose tolerance was essentially normal, the figures for the 3-hour blood test being 95, 155, 166, 59 and 90 mg. per cent. Blood pressure has been constant, averaging 110/70. Red blood cell count was 3.92 million, hemoglobin 80 per cent; white blood cell count, 6800. Blood urea-nitrogen was 19.3 mg. per cent. X-ray interpretation by Dr. Harry Hauser was as follows: "January, 1936. The sella appears rather small but is within the normal limits of measurements, measuring 11 mm. in its anteroposterior and 8 mm. in its cephalo-caudad diameter. The following epiphyses were found to be incompletely fused with their respective diaphyses: distal epiphysis of left acromial process; proximal epiphyses of the left humerus; distal eiphyses of left tibia and fibula; distal epiphyses of both femurs; proximal epiphyses of both tibiae and fibulae; distal epiphyses of right radius and ulna. The epiphyses of the bones of the right and left elbow regions are completely united with their diaphyses. An oval-shaped bone cyst $1\frac{1}{4} \times 3$ cm. is situated in the proximal third of the shaft of the radius. All these un-united epiphyses are due to lack of development based on endocrine disturbances."

This patient presented an unusual and interesting picture. He was unmistakably eunuchoid but the shortness of his stature and the very low B.M.R. indicated decreased function of the pituitary in production of growth and thyrotropic principles. Failure of gonadal development and function seemed most likely also due to hypopituitarism. There must have been some growth principle supplied at some previous time, as evidenced by the length of the extremities relative to height, but it certainly has been less than normal in amount. Silberberg[1] showed that the epiphyseal line in growing guinea pigs undergoes premature closure under the influence of acid extract of anterior pituitary of cattle. It is well known that adequate thyroid function is necessary for normal bone development. It is probably the concurrence of a subnormal output of growth principle, of thyroid and of sex hormones which accounts for the marked retardation of union observed in this patient.

Engelbach[2] found one case of failure of epiphyseal closure at the age of 28 years. This is the oldest such case I have found recorded. McCullagh[3] has seen such a condition in a patient 32 years old.

Summary. There is presented a case of delayed and incomplete union of the epiphyses of the long bones in a eunuchoid patient 44 years of age. Clinical and laboratory findings suggest that this retardation may be due to hypopituitarism with deficiency of sex hormone plus deficiency of growth principle or principles.

† Assay run at the Cleveland Clinic through the kindness of Doctor E. Perry McCullagh.
‡ Assay run at Mount Sinai Hospital of Cleveland through the kindness of Dr. A. M. Young.
[1] Silberberg, M.: Proc. Soc. Exper. Biol. & Med. 32: 1423. 1934-5.
[2] Engelbach, William: Endocrine Medicine, Vol. III, p. 185, Charles C Thomas, Springfield, Ill., 1932.
[3] McCullagh, E. Perry: Personal communication.

EXCRETION OF GONADOTROPIC SUBSTANCE IN POLYCYTHEMIA VERA*

S. LEON ISRAEL AND THEODORE H. MENDELL

The current interest[1, 2] in the relationship of polycythemia to the hypophysis and the structures of the diencephalon (hypophyseal stalk and hypothalamus) stimulated the present study of the urinary excretion of the gonadotropic hormone in polycythemia vera. Although there is no evidence available which specifically indicates the presence of either hypo- or hyperfunction of the pituitary gland in polycythemia vera, there are certain experimental and clinical data which suggest that the hypophysis or its neighboring structures may play a rôle in the etiology of polycythemic states. Briefly stated, the following facts may be correlated in this regard. Polycythemia is part of the syndrome of basophilic adenoma of the anterior pituitary gland.[3] Expanding intracranial lesions in the region of the hypophysis are frequently associated with polycythemia.[2] Castration, resulting in a temporary over-activity of the anterior hypophysis, is accompanied by a decrease in the blood volume.[4] The latter is caused by a decreased plasma volume and, thus, gives rise to a relative polycythemia.

Despite the fact that the demonstration of gonad-stimulating hormone in the urine is only a relative indicator of hypophyseal function, this study was nevertheless undertaken in the hope that results consistently pointing in one direction might be helpful in an understanding of the unknown etiology of polycythemia vera. For that reason, the finer methods of urinary assay for the gonadotropic principle[5] were not employed and the precipitation-test of Zondek[6] was used. Experience with the latter method has shown that normal individuals, excepting those of advanced years[7] and women just prior to ovulation time,[8] never excrete demonstrable quantities of the gonadotropic principle.[9]

Method. Sixty cc. of a freshly-voided, morning sample of urine was filtered, acidified, shaken with 240 cc. of 96 per cent alcohol for 5 minutes, allowed to stand at room temperature for 24 hours and centrifuged. The precipitate was shaken with 30 cc. of ether for 5 minutes (to remove estrogenic substance), centrifuged and the supernatant fluid discarded. The precipitate was dried by evaporation, shaken with 15 cc. of distilled water for 10 minutes, centrifuged and filtered. The aqueous filtrate, containing the gonadotropic hormone, was assayed on 5 infantile mice. Each mouse received 0.5 cc. of the filtrate 3 times daily for 2 days. Vaginal smears

* From the Department of Gynecology and Medicine, Mount Sinai Hospital, Philadelphia, Pa.

[1] Dodds, E. C., and R. L. Noble: Nature **135:** 788. 1935.
Dodds, E. C., J. M. Hills and R. L. Noble: Lancet **1:** 1099. 1935.

[2] Barker, H. W., and W. McK. Craig: Proc. Staff Meet., Mayo Clin. **11:** 548. 1936.

[3] Cushing, H.: Bull. Johns Hopkins Hosp. **50:** 137. 1932.

[4] Friedlander, M., N. Laskey and S. Silbert: Endocrinology **20:** 329. 1936.

[5] Katzman, P. A., and E. A. Doisy: J. Biol. Chem. **106:** 125. 1934.
Freed, S. C., and O. Hechter: Endocrinology **20:** 396. 1936.

[6] Zondek, B., and C. van Eweyk: Klin. Wchnschr. **9:** 1437. 1930.

[7] Zondek, B.: Ztschr. f. Geburtsh. u. Gynäk. **101:** 403. 1932.
Hamburger, C.: Klin. Wchnschr. **12:** 934. 1933.
Leonard, S. L., and P. E. Smith: Am. J. Physiol. **108:** 22. 1934.
Lassen, H. C. A., and E. Brandstrup: Acta obst. et gynec. Scandinav. **14:** 89. 1934.

[8] Kurzrok, R., I. J. Kirkman and M. Creelman: Am. J. Obst. & Gynec. **28:** 319. 1934.

[9] Mazer, C., and L. Goldstein: Clinical Endocrinology of the Female. W. B. Saunders and Company, Philadelphia, 1933.

were made every 12 hours and the animals were killed 100 hours after the first injection. A positive reaction was recorded if the ovaries of at least 3 of the 5 animals showed ovarian stimulation (follicle growth or corpus luteum formation) and if the accompanying vaginal reaction denoted estrus. Control experiments included observations on uninjected mice and on mice injected with known positive urine samples (from menopausal or pregnant women).

Observations. Six patients with polycythemia vera were studied. Three were males and 3 were females. The ages ranged from 24 to 55 years. The duration of the disease was more than 4 years in each instance. The diagnosis of polycythemia vera was established for each patient by blood count and hematocrit determination (table 1). It is interesting to note that the spleen was palpable in 4 instances and that, despite the prevalent opinion to the contrary, hypertension (Giesbock's syndrome) coexised in 2 of the 4 patients. None of the group of 6 patients had a demonstrable quantity of the gonadotropic principle in the urine by the method described above.

Discussion. Employing a method of assay for the presence of gonadotropic principle in the urine which is known to detect no demonstrable quantities of the hormone in normal individuals, with certain exceptions,[7, 8] we were unable to show an

TABLE 1. SIGNIFICANT DATA CONCERNING 6 PATIENTS WITH POLYCYTHEMIA VERA, NONE OF WHOM EXCRETED DEMONSTRABLE QUANTITIES OF GONADOTROPIC SUBSTANCE IN THE URINE.

	1	2	3	4	5	6
Sex	F	F	F	M	M	M
Age	24	44	41	36	55	42
Blood pressure	150/100	130/80	164/100	130/85	120/90	140/90
Spleen palpable	Yes	No	Yes	Yes	Yes	No
R B C	7,920,000	7,470,000	7,300,000	9,600,000	10,000,000	7,800,000
Hb % (Sahli)	117	147	123	145	144	180
Hemocrit (% cells)	75	70	90	70	78	72.2
Gonadotropic Hormone	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

excessive excretion of the principle in a small series of patients with polycythemia vera. Despite the small number of patients observed, we feel that the consistently negative findings challenge the conception that hyperfunction of the anterior hypophysis has a direct etiological bearing to polycythemia vera. However, these results do not militate against the possibility of a disturbance in the water metabolism center (hypothalamus and hypophyseal stalk) in the causation of relative polycythemia. The value of this report, we hope, lies in the stimulation of further interest in the subject of polycythemia and disturbed water metabolism.

Summary. The urinary excretion of gonadotropic substance was studied, employing the method of Zondek, in 6 patients with polycythemia vera in an attempt to evaluate the currently-discussed relationship of the disease to the hypophysis. No significant data were uncovered by the investigation, inasmuch as none of the 6 patients showed a demonstrable (excessive) quantity of the hormone in the urine. The absence of urinary gonadotropic principle in patients with polycythemia vera has no bearing on the possible etiological relationship of the water metabolism center in this disease.

We are indebted to Dr. Thomas Fitz-Hugh, Jr., Chief of the Section on Hematology, Medical Clinic, Hospital of the University of Pennsylvania, for his coöperation in supplying us with cases for this study.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
I. Andrussier
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

The type and degree of gonadal stimulation induced in hypophysectomized male rats parabiotically joined with castrated, cryptorchid and normal partners. Cutuly, E., D. R. McCullagh and Elizabeth Cutuly. To appear in this Journal.

Varying degrees of stimulation occurred in the testes of: a) 5 of 6 hypophysectomized rats joined parabiotically with castrated partners; b) 1 of 4 hypophysectomized rats joined with cryptorchid partners; c) 2 of 4 hypophysectomized rats joined with normal partners. Greater stimulation of the testes occurred in hypophysectomized parabionts when the partners were castrates than when the partners were normal or cryptorchid. The testes of hypophysectomized rats joined with cryptorchid or normal animals were but slightly stimulated. The results indicated, a) that the hypophyses of castrated rats produced or liberated a greater amount of gonad-stimulating hormone than normal hypophyses and, b) that the hypophyses of cryptorchid rats did not produce or liberate more gonad-stimulating hormone than did normal glands. A purely gametokinetic effect which has been said to occur in hypophysectomized female rats joined with castrated partners did not occur in hypophysectomized male rats joined with castrated male twins. In all instances in which gonadokinetic effects occurred in the testes of hypophysectomized rats, whether the partner were castrated, cryptorchid, or normal, it was observed that there was a parallel stimulation of the germinal and interstitial cells. The histological appearance of the interstitial cells proved to be a more reliable criterion of the degree of interstitial cell activation than did the weight or microscopic appearance of the accessory sexual organs.

The effect of female sex hormones upon the oxygen consumption rate of normal rats, and upon the tolerance to desiccated thyroid. Danforth, D. N., R. R. Greene and A. C. Ivy. To appear in this Journal.

Large doses of the following hormones were administered daily to normal rats: estrone, estriol, emmenin, progesterone and the anterior pituitary factor of pregnancy urine. None of them were found to have a significant effect upon the oxygen consumption. After a week of such treatment the daily administration of desiccated thyroid was begun. The rise in oxygen consumption which occurred in control animals was found to be definitely inhibited by treatment with the hormone preparations mentioned above. The results obtained here may explain in part the increased tolerance to desiccated thyroid which occurs during pregnancy.

Tuberculosis of suprarenal glands (Addison's disease). With Report of 7 Verified Cases. Feinblatt, H. M., and B. Alpert. To appear in this Journal.

A study of 7 verified cases of tuberculosis of the suprarenal glands emphasized the insidious onset of this type of Addison's disease. All 4 cardinal symptoms were present in 3 cases, none in 1. There was pigmentation in 3 of the 7 cases, asthenia in 6, gastro-intestinal distress in 6 and abnormally low blood pressure in 4. All 7 patients were free from pulmonary tuberculosis. Five had no tuberculous lesions elsewhere, 1 had tuberculosis of the pituitary

gland, and 1 tuberculosis of the vertebrae. The duration of the disease as evidenced by symptoms varied from 2 weeks to 8 years.

The interrelationship of the pituitary sex hormones in ovulation, corpus luteum formation, and corpus luteum secretion in the hypophysectomized rabbit. Foster, M. A., Ruth C. Foster and F. L. Hisaw. To appear in this Journal.

Hypophysectomy in the rabbit resulted in a progressive loss of function of the corpus luteum in about 5 days; large, medium and small follicles became atretic and degenerated by the end of 2, 15 and 30 days respectively. Primary follicles persisted for as long as 90 to 100 days. Vacuolar degeneration of the interstitial cells began at 30 days and was completed by 55 days. Follicle-stimulating extracts of the pituitary arrested follicular atresia and when injected within 3 days after hypophysectomy prevented it. Ovulation of hypophysectomized rabbits by a single intravenous injection of pituitary extracts was followed within a few days by atrophy of the granulosa with little or no development of lutein tissue. When ovulation was followed by treatment with F.S.H. extracts alone the ruptured follicle failed to develop into lutein tissue and the uteri remained in an oestrous condition. However, after administration of L.H. or a combination of the two hormones functional corpora lutea were formed as shown by a maximal pseudopregnancy reaction in the uterus.

The effect of adrenalectomy and thyroidectomy on ketonuria and liver fat content of the albino rat following injections of anterior pituitary extract. Fry, Edith G. To appear in this Journal.

Adrenalectomy completely suppressed the excretion of acetone bodies by the fasted albino rat following injections of anterior pituitary extract, whether the animals were maintained after operation on physiological saline or on active extracts of the adrenal cortex. The presence of cortical tissue, however, permitted the normal ketogenic response to the extract. Ablation of the adrenals also prevented fatty infiltration of the liver observed in normal rats upon injections of anterior pituitary extract. Demedullation of the adrenals did not prevent accumulation of liver fat in response to the same extract. Thyroidectomized rats excreted large amounts of acetone bodies following pituitary extract whether tested shortly after operation or 6 weeks later. There was no correlation between the time elapsed since removal of the gland and the ketonuria elicited. Phloridzin injections of normal and thyroidectomized rats produced a similar degree of ketonuria.

The effects of thyroidectomy, castration, anterior lobe administration and pregnancy upon experimental diabetes insipidus in the cat. Ingram, W. R., and C. Fisher. To appear in this Journal.

The observation previously made, that thyroidectomy may somewhat reduce but does not abolish the polyuria produced by lesions of the supraoptico-hypophyseal system in cats, has been confirmed in 4 other cats. Feeding desiccated thyroid to such animals elevates the water exchange to equal or exceeding the level existing before thyroidectomy. Castration performed on 4 cats with diabetes insipidus failed to diminish the polyuria, and in one of these the water exchange increased after ovariectomy. Administration of a crude anterior lobe preparation, while ineffective in producing polyuria in normal cats, may increase the water exchange in cats with a tendency to diabetes insipidus. It is not invariably effective, however, and cats already displaying marked polyuria may not respond to such treatment. Cats operated in the early stages of pregnancy may develop marked polyuria which persists, with some indication of diminution immediately before parturition. In the few cases available at present, parturition has been apparently abnormal, has not been completed and has ended fatally for the mother cat. This has prevented observation as to the course of the polyuria after parturition.

Changes in the relative amounts of the follicle-stimulating and luteinizing hormones in the hypophysis of the female rat under varying experimental conditions. Leonard, S. L. To appear in this Journal.

By use of the augmentation reaction it is possible to follow the changes in the relative amounts of F.S.H. and L.H. in the adult female rat hypophysis after castration and after oestrin treatment in the castrates. It was found that castration greatly increases the F.S.H. but only slightly increases the L.H. Oestrin treatment in the castrated female lowers the L.H. level below that found in the normal female but lowers the F.S.H. level to a point just above that of the normal rat. Oestrin treatment in immature female rats decreases the response to

injected urinary F.S.H. (menopause urine) but not the response to urinary L.H. (pregnancy urine). The depletion of the L.H. in the immature rats hypophysis by oestrin will account for the decreased response to injections of urinary F.S.H.

Some factors influencing the vitalization of the ovarian graft and the production of sex hormones in the male rat. Pfeiffer, C. A. To appear in this Journal.

Some of the factors which cause the varied viability of ovarian transplants and the diminution of the male hormone encountered under certain experimental conditions are analysed. Strain differences of host and donor and the amount of lutein tissue in the ovarian graft may be important in the viability of the ovarian transplant. However, these factors have no suppressive action upon the male hormone produced by the male host. Increased body temperature, inanition and toxicity all affect the production of male hormone and are listed in the order of increasing effect. There is a marked destruction of the germinal epithelium from the increased body temperature and by sublethal doses of diphtheria toxin. However, the method of sloughing is strikingly different in the 2 cases. The first is identical with that found in the case of local application of heat to the scrotum, and the second resembles very much that occurring after vitamin E deficiency except the effects are rather immediate. Recovery is possible in all cases.

The effect of complete and partial hypophysectomy in adult albino rats on water, chloride, sodium, potassium and sulfur metabolism. Sandberg, Marta, D. Perla and Olive M. Holly. To appear in this Journal.

The water, chloride, sodium, potassium and sulfur metabolism of completely and partially hypophysectomized rats was studied. In the male rats pronounced polydipsia and polyuria occurred after hypophysectomy, and was still present 10 weeks after operation. In the female rats water intake and output returned to normal after 5 weeks. In the partially hypophysectomized animals the rise was much less pronounced and persisted only 3 weeks. Urinary chloride excretion in the male rats rose from 43% of the intake during the control period to 86% during the first 3 weeks after hypophysectomy, and was still 68% 10 weeks after operation. In the females the increase—from 51% of the intake to 68%—lasted only 3 weeks. It dropped to normal during the next 2 weeks, and even to below normal—33%—during the 6th to 10th week after operation. In the partially hypophysectomized males there was little change in the urinary excretion. Fecal chloride excretion decreased with the diminished intake in all animals. The urinary sodium and potassium excretion followed closely that of the chloride ion; expressed in percentage of intake it was practically the same as that for chloride. In the male rats total urinary sulfur excretion increased from 17% of the intake during the control period to 48% during the first 5 weeks after hypophysectomy, and was 36% 10 weeks after operation. The rise in the excretion of inorganic sulfates, expressed in percentage of the total urinary sulfur excretion returned to normal after 3 weeks. Neutral sulfur excretion increased. The percentage of the fecal sulfur excretion was unchanged, though absolute values decreased with the diminished intake. In the female rats the excretion of total urinary sulfur and of inorganic sulfates returned to normal 3 weeks after operation and dropped below normal after that. Neutral sulfur excretion increased and fecal sulfur excretion remained unchanged. In the partially hypophysectomized rats there was hardly any change in sulfur metabolism.

The metabolism of water, chloride, potassium, sodium, calcium, magnesium and phosphorus in suprarenalectomized rats. Sandberg, Marta, D. Perla and Olive M. Holly. To appear in this Journal.

The water, chloride, potassium, sodium, calcium, magnesium and phosphorus metabolism of suprarenalectomized rats untreated, or maintained on 0.5% NaCl solution, was studied. Water intake remained constant after suprarenalectomy, but increased during NaCl treatment. The urine volume increased. Urinary chloride excretion increased, but the mechanism for chloride storage was not injured by suprarenalectomy, since a drop in fecal excretion counteracted this rise, so that retention in percentage of intake remained constant. During the period of NaCl treatment, retention decreased considerably, due to diuresis. The absolute value of the potassium excreted in the urine showed little change, but there was a drop in the percentage of potassium retained. Urinary sodium excretion increased, but the concentration of sodium per 100 cc. of urine decreased markedly. Calcium and magnesium retention remained unchanged. Urinary as well as fecal phosphorus excretion increased slightly.

Production of estrogenic hormone by a transplantable ovarian carcinoma. Strong, L. C., W. U. Gardner and R. T. Hill. To appear in this Journal.

A spontaneous ovarian carcinoma was found in a multiparous female mouse. Several transplant generations have been made into both normal and castrate males and females of the same strain. In females and spayed females constant oestrous obtains soon after a transplant is made. In males a good estrogenic response is obtained on the mammary glands. The tumor does not grow in all inoculated animals, and in some few cases growth does not bring about a constant oestrous condition in females. Further details of this peculiar physiology are being worked out.

ADRENALS

The demonstration of hormones in unicellular animals. Adrenalin in Paramecia (Ueber den Nachweiss von Hormonen in einzelligen Tieren. Adrenalin in Paramaecium). Bayer, G., and T. Wense, Arch. f. d. ges. Physiol. **237:** 651. 1936.

The dialysate of extracts of Paramecia exerts an epinephrin-like action on the frog's heart and on the rabbit's intestine. The active principle is readily oxidized and is destroyed by ultraviolet light.—A. G.

Modifications in the hypertensional effect of adrenalin caused by colloidal substances (Modifications apportées par certains produits colloïdaux à l'action hypertensive de l'adrénaline). Broun, D., and A. Beaune, Compt. rend. Soc. de biol. **120:** 1202. 1935.

Adrenalin mixed with a colloidal lipoid (1 or 0.5% pseudo-solution of lecithin or cephalin) produced a relatively smaller, less abrupt, but longer sustained rise in blood pressure of atropinized dogs than did adrenalin alone; in addition, tachycardia was suppressed. Records for only 2 dogs are included. Colloidal metals are said to have the same effect as lipoid sols.—E. Cutuly.

Adrenal sympathetic syndrome with unusual variations in cardiac rhythm. Report of a case. Burgess, A. M., G. W. Waterman and F. B. Cutts, Arch. Int. Med. **58:** 433. 1936.

A case is reported of pheochromocytoma of the right adrenal gland with a typical clinical picture, the adrenal sympathetic syndrome and death after operative removal of the tumor. No improvement was observed after roentgen treatment. A study of the heart during the paroxysms of arterial hypertension showed with the onset of the hypertension a slowing of rate with a noticeable increase in the loudness of the sounds and an irregularity of rhythm associated with migration of the pacemaker. It seems probable that many of these changes constitute the cardiovascular reaction to sympathetic stimulation caused by the discharge of large amounts of epinephrin from the tumor into the circulating blood. In view of the striking clinical picture which these patients present, it is to be hoped that in the future the diagnosis may be suspected early, the condition investigated thoroughly and early operation undertaken. In the more obscure cases the authors believe that a suspicious clinical picture, with characteristic changes of blood pressure will justify exploratory operation if the diagnosis cannot be made otherwise, in view of the importance of early diagnosis and the possibility of cure by surgical means.—I. B.

A critical review of 822 operations on the adrenal sympathetic system with special reference to essential hypertension. Crile, G. W., Illinois M. J. **70:** 115. 1936.

In cases of arterial hypertension associated with other diseases due to a pathologic physiology of the adrenal sympathetic system adrenal sympathectomy results in a disappearance of hypertension together with the disease with which it is associated. Crile remarks that it is still too early to offer any statement regarding post-hospital results, although the clinical results during the post-operative stage in the hospital are better than those secured by former procedures.—I. B.

The role of vitamin C in animals resistant to scurvy: insulin and adrenaline. Daoud, K. M., and M. A. S. El Ayyadi, Biochem. J. **30:** 1280. 1936.

The total amount of ascorbic (cevitamic) acid in the livers and suprarenals of adult rats is not reduced by placing them on a scorbutic diet; the urinary excretion of the vitamin decreases, especially at first. The amount present in the liver, in rats on a scorbutic diet, is not affected by starvation or by adrenalin injection, and is increased by insulin: starvation increases and adrenalin decreases the urinary excretion. The extent of the hyperglycemia and glycosuria produced by adrenalin in such animals may be increased by pretreating them with ascorbic acid injections.—D. L. T.

The physiology of pregnancy and the fetus. The permeability of the placenta to hormones and the hypotensor action of fetal adrenals (Contributo alla fisiologia della gravidanza e del feto. Ricerche sulla permeabilità della placenta agli ormoni e sull' azione ipotensiva dei surreni di feto). Falaschino, A., Biochim. e terap. sper. **23:** 289. 1936.

The injection of adrenalin, extracts of adrenals from human fetuses and guinea pigs, acetylcholine and histamine into the circulating blood of the rabbit fetus cause in the mother the characteristic effects on arterial pressure, proving that the hormones and histamine pass through the placenta. Extract from fetal adrenals has a hypotensor action.—A. E. M.

The action of hormones on digestive enzyme secretion. The influence of the adrenals on the amylolytic, lipolytic and proteolytic power of pancreatic secretion (Azione degli hormoni sulla secrezione dei fermenti digestivi. Influenza delle capsule surrenali sul poter amilolitico, lipolitico e proteolitico del succo pancreatico). Ferrari, R., Arch. di fisiol. **36:** 47. 1936.

Adrenalin injected into the dog at a rate of 0.02 mg. per kg. inhibits the pancreatic secretion and reduces the enzymatic activity to a fraction of the normal value. Adrenalectomy causes an increase of pancreatic flow until death occurs. The amylolytic strength is increased 3-5 times, the lipolytic and proteolytic activity 2 times.—A. E. M.

The adrenal glands. A clinical and pathologic study. Hall, E. M., and L. Hemken, Arch. Int. Med. **58:** 448. 1936.

In performing routine necropsies during the past 6 years the authors collected 39 instances of pathological changes in the adrenal glands. These were studied with a view to correlation of the clinical symptoms with autopsy findings. Adrenal hemorrhage occurred in 12.8% of the 39 cases. In 2 persons the hemorrhage was massive and occurred during the course of an acute infectious disease. Clinical signs of adrenal insufficiency were present in only 1 instance. In 2 other cases hemorrhage of moderate degree occurred in the adrenal glands as a terminal complication (in a case of carcinoma of the prostate and in 1 of myelogenous leukemia). There is liability to sudden death, spontaneous or postoperative, in chronic adrenal insufficiency. Death may be sudden and unforeseen, but is most likely to happen when there is a sudden demand for increased adrenal function. This is well illustrated in 5 cases. It is probable that life could have been prolonged in 4 of these if the operation had been deferred or modified. In each of these patients there were signs and symptoms suggestive of chronic adrenal dysfunction. It is evident from an analysis of these records that there is need of additional criteria in establishing the presence of adrenal lesions in the living patient. Not infrequently hypotension or other signs which the physician associates with hypoadrenia may be found during the course of a physical examination in persons who come to the physician with some ailment requiring surgical intervention. Response to injections of extract of adrenal cortex, accompanied by the oral administration of a sodium salt and frequent blood pressure readings, may aid in the diagnosis in cases in which the symptoms are indefinite and pigmentation is absent.—I. B.

The identity in action of the hormone of the adrenal medulla and synthetic adrenalin (Zur Frage der Wirkungsgleichheit des Nebennierenmarkhormones mit dem synthetischen Adrenalin). Kahlson, G., and O. Mertens, Arch. f. d. ges. Physiol. **237:** 699. 1936.

A comparison was made of the blood pressure, blood-flow through the splenic and femoral veins and the cardiac output of anesthetized dogs after stimulation of the nerves to the adrenals and after injection of synthetic epinephrin. The results were identical in both cases, demonstrating the identity in action between synthetic epinephrin and the hormone of the adrenal medulla.—A. G.

Influence of cortin and of epinephrin on the hypophysis, gonads and adrenals of rats (Untersuchungen über den Einfluss von Cortin-Degewop und von Suprareninchlorid auf die Struktur der Hypophyse, der Keimdrüsen und Nebennieren von Ratten). Lippross, O., Endokrinologie **18:** 18. 1936.

Prolonged treatment of juvenile white rats with cortin (Degewop) or with suprareninchloride (Bayer) did not result in any characteristic morphological change in the hypophysis, adrenals or gonads.—W. J. A.

Effect of sex hormones on the vitamin C content of liver and suprarenals in guinea-pigs (Einfluss der Sexualhormone auf den C-vitamingehalt der Nebennieren und der Leber von Meerschweinchen). Mosonyi, J., Ztschr. f. physiol. Chem. **242:** 158. 1936.

The injection of estrogenic preparations reduces the amount of ascorbic (cevitamic) acid in the suprarenals and liver of male and female guinea-pigs by 30-40%, while male hormone preparations have a similar action, but only in male animals.—D. L. T.

Compensatory hypertrophy of the adrenals and standardization of adreno-cortical hormone on rats (Das Zustandekommen der kompensatorischen Hypertrophie der Nebennieren und Beiträge zur Standardisierung des Nebennierenrindenhormons an Ratten). Reiss, M., J. Bálint and V. Aronson, Endokrinologie **18:** 26. 1936.

In hypophysectomized rats removal of 1 adrenal is not followed by compensatory hypertrophy of the remaining adrenal. After incomplete hypophysectomy there is frequently a greater compensatory hypertrophy of the remaining adrenal than in animals with the hypophysis intact. Completely hypophysectomized rats always die after removal of both adrenals; but by administration of cortical hormone they may be kept alive for months. Such completely hypophysectomized and adrenalectomized rats may therefore be employed as test animals for the standardization of cortical hormone.—W. J. A.

Changes in utilization of fat in adrenalectomized rats (Veränderungen des Fettumsatzes epinephrektomierter Ratten). Reiss, M., H. Epstein, F. Fleischmann and L. Schwarz, Endokrinologie **17:** 302. 1936.

Total body fat of rats falls after adrenalectomy. Total cholesterin is decreased 4 days after operation and increased 14 days after. In starvation adrenalectomized rats die earlier, at a higher weight and a higher total fat content than normal animals. Their cholesterin content is lessened. Adrenalectomized rats are incapable of being fattened with fatty foods. After 15 days of heavy feeding there is little deposited fat whereas normal animals, so fed, have greatly increased fat depots.—W. J. A.

Changes of the sodium and chloride content of tissue after adrenalectomy (Anderungen des Natrium—und Chlorgehaltes der Gewebe nach Nebennierenentfernung). Rigler, R., Arch. f. exper. Path. u. Pharmakol. **181:** 127. 1936.

The determination of the Na and Cl as well as water content of the various tissues of adrenalectomized and sham-operated control rats was made by the author. The electrolyte values were lower in the adrenalectomized group than in the control group; values for the content of the liver remained constant for the two groups. The water content of the tissues investigated was slightly increased in the adrenalectomized rats.—R. Cleveland.

Relation of adrenal glands to experimental pancreatic diabetes. Rogoff, J. M., and H. W. Ferrill, Proc. Soc. Exper. Biol. & Med. **34:** 100. 1936.

Partial adrenalectomy, or adrenal denervation, with or without ablation of the medulla, did not prevent or diminish experimental diabetes in dogs. These findings are the reverse of those reported earlier. Incidentally, pancreatectomized dogs maintained with insulin showed a decreased epinephrine output, even when there had been no interference with the adrenals.—M. H. F.

Mineral exchange between plasma and erythrocytes in Addison's disease (Mineralaustauschvorgange zwischen Plasma und Erythrocyten beim Addison). Schmitt, F., and W. Basse, Arch. f. exper. Path. u. Pharmakol. **181:** 581. 1936.

Ion distribution between plasma and erythrocytes of a patient with Addison's disease was studied. The Cl values were 100 and 29 millimols respectively; Na, 140 and 20; K, 5.1 and 90; and Ca, 2.7 and 0.3. Analyses were made on the same patient following cortical extract therapy accompanied by the administration of NaCl. Under this treatment the Cl values were 103 and 46.4 millimols in the plasma and erythrocytes respectively; Na values were 124 and 40; K, 4.9 and 5.7, and Ca. 2.4 and 0.6. Where the cortin therapy was accompanied by administration of $NaHCO_3$ the Cl values were 108 and 64 millimols in plasma and erythrocytes respectively, Na, 140 and 32.6; K, 4.1 and 45.5 and Ca, 2.37 and 0.78. The authors reported the Cl and Na losses in Addison's disease much greater in the erythrocytes than in the plasma. Cortin and NaCl therapy shifted the Na content of the plasma to the erythrocytes somewhat; $NaHCO_3$ increased the Na content of the total blood. Ca showed only a slight tendency to

a decrease in the plasma and an increase in the erythrocytes. Clinically it was shown that cortin and salt therapy increased the NaCl of the tissues and also made possible a larger retention of fluid in these tissues. No marked effect on the acid-base balance was reported. Smaller doses of cortin were effective when the salt treatment accompanied the administration of the cortin.—R. Cleveland.

On the biological assay of the cortical hormone by the survival method, in adrenalectomized young rats, and on the influence of the salt content of the hormone extract. Schultzer, P., J. Physiol. **87:** 222. 1936.

Young rats (77) had 100% mortality after bilateral adrenalectomy and kidney upper pole decapsulation when kept on Gudjunsson's No. 4 diet. The average survival period of 5.7 days was prolonged to an average of 7.7 days by injection of 1 cc. of 0.9% saline daily (30 rats) and to 9.3 days by 1 cc. twice daily (11 rats). Of 146 young rats (50 gm.) treated with corticoadrenal extract (Ecortan 110) about 54% survived during the injection period of 21 days. On stopping injection these 79 animals died in 2 to 28 days. By plotting percentage survival for the various doses used it was found that a sharp drop in survivals occurred when the amount injected was less than 12.5 gm. of adrenal gland daily. With another series of 17 animals a second preparation (Ecortan 156) gave 100% survival for 21 days when each rat received the equivalent of 50 gm. daily, but only 60% survival when 25 gm. were given per rat per day. The method is proposed for assay of adrenal cortical hormone, using a formula to determine relative potencies.—R. L. Z.

Revival from insufficiency and maintenance of adrenalectomized dogs with low serum sodium and chloride levels. Swingle, W. W., W. M. Parkins and A. R. Taylor, Proc. Soc. Exper. Biol. & Med. **34:** 75. 1936.

By injections of adrenal cortical extract it was possible to revive dogs in collapse from adrenal insufficiency and restore the normal blood volume without raising the serum sodium and chloride from their low, preinjection levels. Moreover on a diet relatively free from such elements, dogs could be maintained for 2 weeks or more by suitable injections of cortical extracts with continued low serum sodium and chloride values. Similar results were obtained in adrenalectomized dogs which had had serum sodium and chloride depleted by intraperitoneal injections of isotonic glucose. By withholding or injecting the cortical extract in such animals it was possible to shift fluid from blood to tissues, or, vice versa, without raising the serum electrolytes. It is apparent to the authors that the clinical conditions of the animals considered is more related to shifts in body fluid to and from the blood rather than to the serum electrolyte levels.—M. H. F.

Effects of estrus and certain gonadotropic hormones on life-span of adrenalectomized animals. Swingle, W. W., W. M. Parkins, A. R. Taylor and J. A. Morrell, Proc. Soc. Exper. Biol. & Med. **34:** 94. 1936.

Five adrenalectomized dogs in heat were maintained in good health without cortical extract for 40 to 60 days, during which time blood pressure and blood chemistry were normal. Artificial heat, induced in a bitch by the injection of extract of menopausal urine, permitted good health in an adrenalectomized animal for 25 days. Similar results were obtained by the injection of a growth hormone preparation into adrenalectomized animals, although it is noted that this preparation contained considerable amounts of both thyrotropic and gonadotropic factors. —M. H. F.

Cortico-adrenal insufficiency and potassium metabolism. Truszkowski, R., and R. L. Zwemer, Biochem. J. **30:** 1345. 1936.

A method for determining potassium colorimetrically as argenticobaltinitrite in 0.5 gm. of whole blood or muscle is described. In adult male cats, the amount of potassium per 100 cc. whole blood rises from 21.4 mg. beforehand to 36.4 mg. on the third day after bilateral adrenalectomy, and thereafter fluctuates without attaining higher levels; even distribution between cells and plasma is maintained throughout. In skeletal and cardiac muscle, the amount of potassium per 100 gm. dry weight increases in insufficiency, but this is more than compensated for by increased water content of the tissues. The absence of progressive increase in blood potassium with advancing insufficiency symptoms is ascribed to anorexia and reduced potassium intake. Maintenance of blood potassium at the levels described will produce, in normal cats, all the symptoms of adrenal insufficiency.—D. L. T.

The relation between vitamin B_2 and the hormone of the adrenal cortex (Der Zusammenhang zwischen Vitamin B_2 und dem Hormon der Nebennierenrinde). Verzár, F., and L. Laszt, Arch. f. d. ges. Physiol. **237:** 476. 1936.

A commercially available preparation of the adrenal cortical hormone was administered to a series of 9 adrenalectomized rats on a vitamin-B_2-free diet and on a normal diet. In the absence of vitamin B_2, the hormone was unable to maintain life in the adrenalectomized animals. Flavin phosphoric acid, on the other hand, maintained 10 adrenalectomized animals alive for 10 days.—A. G.

Effects of various corticoadrenal extracts on diphtheria toxin in vivo and in vitro. Zwemer, R. L., and C. W. Jungeblut, Proc. Soc. Exper. Biol. & Med. **32:** 1583. 1935.

An attempt to isolate from the adrenal cortex the factor responsible for resistance to toxins gave evidence that it was to be found in the same fractions as the vital hormone, cortin. Using 46 light colored, 350 gm. guinea pigs it was found that the characteristic skin reaction following intracutaneous injection of diphtheria toxin could be diminished or prevented by previous injection of active adrenal cortex extract. When 2 m.l.d. of diphtheria toxin was given to each of 24 control 250 gm. guinea pigs all died within 72 hours. Another 28 guinea pigs received the same amount of toxin (2 m.l.d.), but mixed with an equal volume of (1 cc.) adrenal cortex extract. Of these 35% survived, while 25% lived longer than the controls; the remaining 40% were unprotected. In a third series of 45 animals weighing 250 gm. the toxin and extract were injected separately. To obtain 50% protection in this series required extract 100 times as strong as that of the previous series in which extract and toxin were mixed before injection. The possibility that lipoidal inactivation of toxin had occurred was discounted by the activity of certain aqueous hormone fractions and the inactivity of fractions containing little cortin and much phospholipin or sterol.—R. L. Z.

ENDOCRINE GENERAL

Inheritance of endocrine disturbances (Zur Vererbung endokriner Störungen). Aschner, B., Wien. Arch. f. inn. Med. **29:** 69. 1936.

Sporadic goiter and hyperthyroidism both are familial and are related. Apparently the same pathological allelomorph may be acted upon to produce either variety of disturbance. Milder forms of intersexuality are definitely hereditary.—W. J. A.

Endocrine obesity. Bulger, H. A., M. Clin. North America **20:** 269. 1936.

The common notion that reduced combustion from thyroid deficiency can explain all obesity has proved a delusion. Bulger points out that hypothyroidism is rarely if ever a primary cause of marked obesity. In treatment, there should be a more conservative use of drugs and a more radical use of diets. Thyroid should not be used unless in the presence of a sluggish mentality and a distinctly low basal metabolic rate. Patients should be made to utilize their stores of fat by an appropriately prescribed diet, characterized by adequate proteins, vitamins and salts and almost no fat, and minimal amounts of carbohydrates.—I. B.

Body build factor in the basal metabolism of boys. Molitch, M., Am. J. Dis. Child. **50:** 621. 1935.

A total of 521 basal metabolic tests were made on 199 boys on whom width-length indexes had been obtained. The boys ranged in age from 10 to 18 years and did not include any with evident endocrine disturbances. Successive determinations were made every 24 hours until not more than a 4% difference was obtained and an average taken. Correlations were made between the basal metabolic rate as expressed in calories per sq. m. and in calories per kg. per hour and the width-length indexes. The author was unable to confirm the work of Lucas and Pryor in that there was no correlation between body build and the oxygen absorption. Higher metabolic rates were not characteristic of the linear type of body build.—M. B. G.

Growth and basal metabolism. Changes in the basal metabolism of children during a year. Nakagawa, I., Am. J. Dis. Child. **49:** 1232. 1935.

The basal metabolism of 26 children, 3 years, 10 months to 11 years, 2 months of age, was studied every other month throughout the year and the following conclusions were reached:

In children there is an increase of about 30 calories in the total amount of heat produced annually. There is a higher increase in girls between the ages of 11 and 12 years due perhaps to the increased rate of growth at puberty. The amount of heat produced does not increase gradually throughout the year but shows seasonal variations, the maximum amount being produced during January and the minimum in July. The amount of heat produced by one child deviates only slightly at different times during the year. The coefficient of correlation between the basal heat production and the weight, height and surface area have been calculated, using 162 sets of data and it has been demonstrated that the weight in the case of children is the most reasonable and practical factor for expressing the basal heat production.—M. B. G.

Research involving weight of tadpoles (Die Methodik des Kaulquappenwägeversuchs). Romeis, B., Endokrinologie **18:** 10. 1936.

The author describes this method of weighing, which involves a series of 3 sieves, in the last of which the tadpole remains during the weight determination on a rapid, specially-devised balance. The relative value of weight determinations as compared with linear measurements is discussed.—W. J. A.

Metabolism of adolescent girls. Basal metabolism and energy exchange. Wang, C. C., M. Kaucher and M. Wing, Am. J. Dis. Child. **51:** 801. 1936.

The basal metabolism of 34 adolescent girls between the ages of 11 and 20 years, varied from 968 to 1,511 calories per 24 hours, with an average of 1,191 calories for the prepubertal group and 1,317 calories for the postpubertal. The averages for calories per kg. per 24 hours were 33.6 for the prepubertal girls and 27.2 for the postpubertal. The values for basal metabolism were correlated with those for height, weight, age and surface area, using both the values in total calories per 24 hours and those in calories per kg. per 24 hours. In the case of the total calories, the highest coefficient of correlation was that with surface area followed closely by that with height and that with weight. When the values in calories per kg. were used, all the coefficients were negative. Of all correlations made between caloric intake and the various factors, that with growth and activity had the highest coefficient.—M. B. G.

Oestrogenic hormone and the mechanism of corpus luteum formation in the rabbit. Bachman, C., Proc. Soc. Exper. Biol. & Med. **33:** 551. 1935.

It was impossible to confirm Hohlweg's report that large doses of estrin (Folliculin-Girard) induced corpus luteum formation in the immature rat. As much as 2 mg. of the crystalline material administered in a single dose to young rats was without effect on the ovaries. The injection of doses of 2 to 15 mg. of this material into isolated rabbits failed to stimulate ovulation or corpus luteum formation. The injected rabbits were mated 12 days following the estrin injections, and ovulation occurred normally in the mated females. These animals were sacrificed on the third day of pseudopregnancy, and the pituitaries weighed. The average pituitary weight of the injected animals was 38 mg., compared with an average of 42 mg. for the pituitaries of non-pregnant animals in the same laboratory. It is concluded that large doses of estrin do not cause luteinisation of either the rat or rabbit ovary, and do not have any marked effect on the weight of the rabbit pituitary.—M. H. F.

Reactions of immature rabbit ovary to gonadotropic extracts. Bachman, C., Proc. Soc. Exper. Biol. & Med. **34:** 33. 1936.

Immature rabbits, 15 to 90 days old, were injected with 2 to 4 U. of sheep A.P. daily for 14 days (subcutaneously) and with 100 U. of urinary A.P.L. intravenously for 14 days. (True vesicular follicles are not found in rabbits before 60 days.) The youngest animals to respond to A.P.L. were 45 day old females. The response consisted of corpus luteum formation in 2 of the 7 treated animals, and the formation of clusters of large, lutein, polyhedral cells in the ovarian stroma of the remaining 5 females, without the appearance of theca luteinisation in either group. In the 60 day rabbits injected with A.P.L. most of the animals showed corpus luteum formation (together with corpus hemorrhagicum formation), although 1 animal showed the stroma changes without significant granulosa luteinisation. The response to the injections of sheep A.P. was essentially the same, except that the first responses were obtained somewhat later (60 day animals) than with P.U., and that the follicular response was somewhat more abnormal. In general, with either kind of injection, the stromal changes were more

prominent when granulosa luteinisation was absent, or abnormal, and vice versa. Moreover, in neither series was theca luteinisation seen. In some of the young animals, the presence of the corpora lutea was not associated with progestational uterine change.—M. H. F.

GONADS

Gonadotropic active substance in the urine of patients with tumors (Gonadotrope Wirkstoffe im Urin von Tumorkranken). Baudler, U., Monatschr. f. Geburtsh. u. Gynäk. **102:** 156. 1936.

The urine of patients with benign and malignant tumors was tested for gonadotropic hormone according to the method of Zondek. In a series of cases including adenocarcinoma and squamous cell carcinoma of the cervix, carcinoma of the vulva, ovaries, mammae, prostate and penis and sarcoma of the trochanter, femur, face and knee, increased amounts of prolan were demonstrated in the urine. There was no appreciable increase in gonadotropic hormones in benign cases as myomata, cervical polyp, endometrial tbc. and prostatic hypertrophy. The excretion of prolan was increased following radiation of malignant tumors. The author suggested that the amount of prolan excreted is proportional to the size, infiltration and degeneration of the tumor. It is questioned whether the prolan excreted by cancer patients is chemically identical with that excreted during pregnancy.—M. R. White.

The ambosexual character of the sex hormones and some consequences derived therefrom (Le charactère ambosexuel des hormones génitales et ses conséquences). Champy, C., Bull. Acad. de méd. **113:** 915. 1935.

Female and male sex hormones are found in either sex. A deficiency may cause disturbances not related to the sexual sphere. Vascular disturbances in the male are occasionally observed as a consequence of estrin deficiency. A case of arteritis in a man of 50 years is reported. Both legs were paralyzed, sensation had disappeared, and the temperature dropped. Amputation seemed to be necessary. Treatment with folliculin brought about a complete remission. Incidentally, the voice of the man changed from a barytone to falsetto.—A. E. M.

Environment and sex in the oviparous oyster Ostrea virginica. Coe, W. R., Biol. Bull. **71:** 353. 1936.

The histological basis for the changes of sex in the Virginia oyster depends on the essential bisexuality of the gonads. At the first breeding season, determination of the sex at various localities showed the percentages of females to vary from 3.07 to 32.81.—E. L.

Sequence of functional sexual phases in Teredo. Coe, W. R., Biol. Bull. **71:** 122. 1936.

The sexual condition of 2,599 individuals of Teredo navalis, grouped according to their sizes and approximate ages, was determined. Protandric bisexuality is characteristic of the early sexual phases with a tendency to alternating unisexuality in later life. Four phases may be completed in a lifetime of a Teredo.—E. L.

Folliculin and carbohydrate metabolism (Folliculine et métabolisme hydrocarboné). Collazo, J. A., and B. Marti, Ann. de méd. **38:** 383. 1935.

Folliculin and similar preparations produce an increase of blood sugar of about 30%, which is concomitant to an increase of lactic acid of 97%.—A. E. M.

The nomenclature of estrus-producing compounds. Committee on Nomenclature of Endocrine Principles, J.A.M.A. **107:** 1221. 1936.

The Committee briefly review the history of research on the estrus-producing compounds. It is pointed out that there are at least 7 naturally occurring estrogenic substances that have been isolated in a crystalline condition. Several of these compounds have received specific names at the hands of their discoverers, and the synonymy has become confused. Among the names in common use are oestrin, estrogen, menformen, thelykinine and folliculin. The term oestrin has come into rather widespread usage, particularly in England. Doisy's terms theelin and theelol have been widely used in America but have had less recognition in England and on the Continent. For reasons set forth at some length it was decided to adopt a system of nomenclature based on the root "estr-"; to retain theelin, theelol and dihydrotheelin for the compounds otherwise known as estrone, estriol and estradiol, respectively; and to adopt the term estrogenic to describe those compounds or extracts which in addition to their other physiologic properties reproduce estrus, and to adopt the noun estrogen as the collective for all the sub-

stances having these properties. A table is included in the report giving the structure, empirical formulas and the common and chemical names of 11 different estrogens. The 3 synonyms in most common use are estrone or theelin, estradiol or dihydroxytheelin, and estriol or theelol.—R. G. H.

Further observations on the oestrogenic activity of synthetic polycyclic compounds. Cook, J. W., E. C. Dodds and W. Lawson, Proc. Roy. Soc. London, s.**B.**, **121:** 133. 1936.

A series of homologues of 9:10-dipropyl-dihydroxy-1:2:5:6-dibenzanthracene have been prepared, but are of lower or zero activity. Similar derivatives of phenanthrene, chrysene, and 1:2- or 2:3-benzanthracene have been studied, the majority being inactive. The first synthetic estrogenic substance known, 1-ketotetrahydrophenanthrene, loses activity when reduced to the alcohol, although among natural estrogens the alcohols are more potent than the ketones. The methyl ether of estrone (theelin) has 2-10% of the activity of the parent substance; it has hitherto been regarded as quite inactive. The dipropyldihydroxydibenzanthracene abovementioned replaces estrone in all its known biological effects, including sensitization of the endometrium to progesterone action, the pathological effects of continued excessive administration, the control of plumage in capons, etc.—D. L. T.

A biological assay of "International Standard" estrin and of certain commercial preparations. D'Amour, F. E., and R. G. Gustavson, J. Pharmacol. & Exper. Therap. **57:** 472. 1936.

Recently ovariectomized rats were used in assaying the potency of international standard estrin, theelin, amniotin and progynon by the single injection method of Coward and Burn and by the multiple injection method of Marrian. The R.U. (amount necessary to produce a positive reaction in 50% of spayed rats) of international standard estrin was: single injection in oil, 1.3γ; multiple injection in oil, 1.5γ; single injection, aqueous, 3.8γ; multiple injection, aqueous, 0.74γ and multiple injection, emulsion, 0.76γ. The commercial preparations in oil solutions contained the stated amounts of activity but the aqueous preparations contained only 1/6 to 1/7 of their stated biological activity.—E. L.

The gonadotropic factor as an aid to surgery in the treatment of undescended testicle. Deming, C. L., J. Urol. **36:** 274. 1936.

Two young monkeys were treated with gonadotropic hormones. Descent of the testis was brought about at a much earlier period than normal in this form. The author concludes that the gonadotropic factor is a definite aid preceding and following surgical treatment of the undescended testicle. It enlarges the inguinal canal and the cord, and renders the testicle more movable in the monkey. Treatment may be instituted as early as 2 years of age in the human. The earlier the treatment the better are the chances for a normally functioning testicle. The histologic picture in the monkey 6 weeks after the cessation of the administration of the gonadotropic factor persists with negligible change.—J. C. D.

Maldevelopment and maldescent of the testes. Report of treatment with the anterior pituitary-like gonadotropic hormone from the urine of pregnant women. Dorff, G. B., Am. J. Dis. Child. **50:** 649, 1935.

Fourteen boys ranging in age between 6 and 13 years and showing hypoplasia of the genitalia in the form of either maldescent or maldevelopment of the testes were studied and treated. The author divides his patients into two types: the adiposogenital dystrophy and the hypothyroid. Therapy consisted of the injection of anterior pituitary-like hormones from pregnancy urine. Results in both types were: Increase in size of hypoplastic testes; descent and increase in size of undescended testes and increase in size of scrotum, and penis; development of pubic hair; stimulation of epididymis and prostate. The author claims that congenital hernia which frequently accompanied cryptorchidism may correct itself during the process of descent and that the mental aspect of the patient changes. Treatment should be started at any time prior to puberty but better results are noted in patients closer to puberty.—M. B. G.

Treatment of several types of adiposogenital dystrophy in boys. With particular reference to the use of gonadotropic hormone from the urine of pregnant women for their imperfectly developed genitals. Dorff, G. B., J. Pediat. **8:** 704. 1936.

Unusual complications such as torsion, mechanical obstruction and hernia are discussed. It is apparent that the congenital hernia which accompanied an undescended testis situated intra-abdominally or at the internal ring could be corrected when the testis descends into the

scrotum through the stimulation of the gonadotropic hormone. In cases in which there had been a delay in descent, the latter could be initiated by hormone stimulation and the entire inguinal region could usually be brought to maturation. The value of combined therapy (gonadotropic hormone of pregnancy urine, dietary restriction and desiccated thyroid) was shown in that not only was genital development stimulated and maldevelopment corrected but also many of the accompanying deficiencies of a skeletal, somatic or mental nature could be improved.—M. B. G.

Maldevelopment and maldescent of the testes. Further observations on treatment with anterior pituitary-like gonadotropic hormone. Dorff, G. B., Am. J. Dis. Child. **50:** 1429. 1935.

The present paper reports further observations and additional data on 14 boys with maldeveloped or maldescended testes who were treated with anterior pituitary-like hormone from pregnancy urine. Prolonged and intensive treatment is a requisite for a complete result. The testes increase in size only within the physiological limits of boys' age but in a boy who is of pubertal age the enlargement may be beyond that of the normal. Overstimulation may cause precocious development of the external genitalia and the secondary characteristics in young boys. Treatment apparently has no ill effects on other parts of the body such as mammary glands or facial hair or general hirsuties. Cleancut evidence is lacking that the thyroid gland is secondarily stimulated. The osseous development was not further depressed by treatment with anterior pituitary-like hormone.—M. B. G.

The progesterone treatment for dysmenorrhea. Elden, C. A., and K. M. Wilson, Am. J. Obst. & Gynec. **32:** 91. 1936.

Seventeen patients were treated with total doses of 2/25 to 1 Rb.U. of progestin, in one or more doses given in the last few days before expected menses. Either preparations made by them or proluton were used. Of these 47% gave complete relief from dysmenorrhea, and 41.3% no help. The treatment was varied and replaced with inactive oil, or omitted often enough to make the evidence of relief from progestin convincing. No change in menstrual interval, duration, or character of flow was observed.—E. L. S.

The action of testosteron on the growth of the crest of Triton cristatus (Wirkung des Testosteron auf das Wachstum des Kammes von Triton cristatus). Fleischmann, W., and S. Kann, Arch. f. d. ges. Physiol. **237:** 517. 1936.

Injection of testosteron causes growth of the serrated crest which characterizes the male newt during its breeding season.—A. G.

Estrogenic substances in the blood of women. Fluhmann, C. F., Am. J. Obst. & Gynec. **32:** 612. 1936.

The test animal is an adult female castrate mouse, receiving 6 injections of serum in 48 hours, 4.5 cc. total, with post-mortem examination of the vagina 15 hours after the last injection. The graded reaction has been described, consisting of stages of mucification. The sensitivity of the test is to about 1/8 M.U. or 3 U. per 100 cc. of serum. The doses responsible for the graded reaction are in ratios 1:2:4:8:16. The author reports 401 tests on 84 women and 15 prepubertal girls. Estrogenic material begins to be demonstrable at about age 7. In fairly normal cycles the rise in estrogenic substance occurred at about the time of ovulation, with some cycles showing secondary rises at the onset of menses. In 4 cases with anovulatory cycles no generalizations are warranted. The commonest finding in 16 cases with endometrial hyperplasia was a rise of titre at the onset of bleeding. Amenorrheic cases showed cyclic or acyclic curves, or no estrogenic material present. In some cases positive results were obtained after the menopause, or after castration.—E. L. S.

The relation of male sex hormone (testicular hormone) in the female body at the end of pregnancy (Das Verhalten des männlichen Sexualhormons (Testikelhormon) im weiblichen Körper am Ende der Schwangerschaft). Goecke, H., Ztschr. f. Geburtsh. und Gynäk. **112:** 273. 1936.

An investigation to determine the presence and the relationship of the male sex hormone in the female body at the end of pregnancy demonstrated the testicular hormone in 71% of the placentas from female fetuses but was not demonstrated in any placenta from a male fetus. No male sex hormone was demonstrated in the placenta of a male, although large amounts are excreted in the urine of the mother. Testicular hormone was excreted in the urine where boys

were later born but not in the case of female fetuses. The author suggested the possibility of determining sex in utero by an estimation of the testicular hormone in the urine at the end of pregnancy. If there was a male fetus, there was excreted one cockscomb unit of testicular hormone in three liters of urine in at least 80% of the cases.—M. R. White.

Non-effect of estrogenic hormones on mammary gland of hypophysectomized guinea pig. Gomez, E. T., and C. W. Turner, Proc. Soc. Exper. Biol. & Med. **34:** 320. 1936.

The injection of varying amounts of estrogenic material into young, hypophysectomized male and female guinea pigs for 20 to 65 days resulted in some nipple growth, but no growth of alveolar or duct tissue of the mammary gland. These results are in accord with those of Lyons and Pencharz.—M. H. F.

Bioassay of a granulosa cell tumor. Gospe, S. M., Am. J. Obst. & Gynec. **32:** 495, 1936.

The tumor was sarcomatoid. Assay showed 3.2 M. U. per gram net weight.—E. L. S.

Comparative assay of oestrone in the rat and the mouse. Hain, A. M., and J. M. Robson, J. Pharmacol. & Exper. Therap. **57:** 337. 1936.

Under carefully controlled conditions, using the international standard of oestrone and a crystalline benzoate material, the rat unit of oestrone was 0.0033 mg. when it was given in 4 doses in oil and 0.0025 mg. when administered in 10% alcohol. The equivalent figures for the mouse were 0.00009 mg. and 0.00025 mg. There was a considerable decrease in potency when the oestrone was administered either in a single injection or in a course of hourly injections over a period of 12 hours. Full cornification was reached in the rat in 72 hours after the first injection and in the mouse in 76 to 80 hours. Due to the differences in the amounts of estrin necessary to sensitize the uteri of the mouse and rat to oxytocin, the authors believe that abortion in the mouse is not dependent on the estral morphological changes.—E. L.

Assays of hypertrophied prostatic tissue and of urine for estrogenic substances. Hamilton, J. B., C. L. Deming and E. Allen, Proc. Soc. Exper. Biol. & Med. **34:** 193. 1936.

Assays of surgically removed hypertrophied prostates, and of the urine from such patients before and after operation failed to reveal the presence of estrogenic material.—M. H. F.

The chemistry and biology of the pure corpus luteum hormone progesteron (Zur Chemie und Biologie des reinen Corpus luteum Hormons "Progesteron"). Hohlweg, W., and J. Schmidt, Klin. Wchnschr. **15:** 265. 1936.

Alpha and beta progesteron are easily converted from one to another, display identical properties in solution, and each forms a dioxime with a melting point of 244, the products being indistinguishable. These facts are cited in favor of the conception that alpha and beta progesteron are merely polymorphs of the same chemical substance. These two polymorphs were assayed according to the Corner-Allen technique and the Clauberg technique. With both methods no quantitative or qualitative difference could be detected between the alpha and beta forms, and there was no evidence of synergism when the two forms were administered together. These biologic findings support the chemical findings of earlier experiments, and do not support the contention of Fels that the two forms of progesteron are chemically and biologically distinct substances.—M. H. F.

Influence of the central nervous system on the lactating mammary gland of the rat (Influence du système nerveux central sur la mamelle lactante chez le rat blanc). Ingelbrecht, P., Compt. rend. Soc. de biol. **120:** 1369. 1935.

In a group of 10 female rats which had been lactating for at least 48 hours the spinal cord was sectioned between the last thoracic and first lumbar vertebrae, rendering the caudal group of 6 mammary glands insensible; the cranial group of 6 glands remained intact. When the cranial group of glands was covered by a jacket, so that the litter had access to only the caudal group, the young died, and at autopsy their stomachs were empty. If but 2 of the cranial glands were left exposed, lactation continued in all of the glands, innervated or not. Cessation of lactation occurred in all of the glands when the cranial group was jacketed; secretion was reëstablished in both groups when the jacket was removed and sucking on the cranial group was resumed. It is believed that a reflex stimulation originating in the mammary glands passes by way of the cerebrospinal axis and that the response is transmitted through a humoral route.—E. Cutuly.

Artificial stimulation of corpora lutea in rabbit ovaries. (Künstliche Erzeugung von Gelbkörpern in Kaninchenovarien. Kandaratsky, V. S., Zentralbl. f. Gynäk. **60:** 2418. 1936.

Ovarian follicles of rabbits were aspirated. This did not lead to corpus luteum formation but to atresia. Luteinization followed coitus or prolan treatment, the latter leading to the change also in follicles which had not been ruptured artificially. The prolan leads also to further follicle development. The retention of an ovum does not influence the luteinization, the egg eventually disappearing. The formation of corpora lutea is therefore a result of continuing processes operating upon the ovaries.—E. L. S.

The use of progesterone in combating habitual abortion. Kane, H. F., Am. J. Obst. & Gynec. **32:** 110. 1936.

The series of 40 cases treated with corpus luteum includes 26 previously reported in which corpus luteum extract had been used with 4 failures. In the more recent 14 no failures occurred. Thyroid was used in all cases in addition to the progestin, as before. The doses were 1/25 unit of proluton, intramuscularly, alternate days for 10 doses, repeated at 3 week intervals until the end of the 4th month. Two cases who were successful under this treatment undertook pregnancy later, without the therapy but with abortion again as before. Results also suggest that foetal abnormalities are frequent among women who bear children after having aborted previously.—E. L. S.

Seasonal sexual activity and its experimental modification in the male sparrow, Passer domesticul Linnaeus. Kirschbaum, A., and A. R. Ringoen, Anat. Rec. **64:** 453. 1936.

Progressive gonadal changes from October to April were studied for the male sparrow. Minimum gonad size is found in October, November and December. During January and February there is gradual enlargement of the testis, and in March there is active spermatogenesis with sperm production late in the month. Sperm production not ordinarily found until late March can be induced in juvenals and adults by subjecting birds to increased daily light ration in the late fall and early winter months. As early as December 10, after 6 weeks of light treatment, sperm cells were observed in the testes of experimental birds. Elevation in temperature (12° to 23°C.) during October to January appeared not to have any effect on the sparrow testis during the same time that increased light ration induced full testis activity. Reduced light ration in the spring retards the development of the germinal epithelium of the sparrow testis. Since there is not a complete suppression of germ cell activity when birds are given very little daylight, it seems that there are factors beside light operating in producing the seasonal sexual cycle. The juvenal male acquires a black beak during testis enlargement. If testis enlargement is suppressed in the spring, the juvenal retains a light-colored beak.—Authors' Abst., Wistar Bib. Service.

The assay and the effect of testosterone on rats compared with those of other sexual hormones. Korenchevsky, V., M. Dennison and I. Brovsin, Biochem. J. **30:** 558. 1936.

It was found that the rat unit, the smallest amount required to produce 40% increase in prostate weight in young castrate male rats, is 7 to 8 gamma for estosterone, as against 170 gamma for androsterone. Even in large doses, however, testosterone does not completely restore the sexual organs after extensive castration atrophy, and it does not reduce castration-hypertrophy of the hypophysis. It partly restores the atrophied sexual organs of ovariectomized female rats and restores the prostate and the seminal vesicles in their normal proportion, whereas androsterone especially affects the former.—D. L. T.

The assay of transdehydroandrosterone and its effects on male and female gonadectomized rats. Korenchevsky, V., and Marjorie Dennison, Biochem. J. **30:** 1514. 1936.

Transdehydroandrosterone, which occurs in urine and may be prepared from cholesterol, has less than one-fifth of the activity of androsterone in restoring the prostate of castrated male rats; the property of affecting the prostate rather than the seminal vesicles is not as marked as in androsterone, but more marked than in testosterone.—D. L. T.

The small-cystic ovary (Das kleincystische Ovar). Kraul, L., Zentralbl. f. Gynäk. **60:** 28. 1936.

The literature and current ideas are reviewed. The author presents his opinions without data, indicating that the presence of small graafian follicle cysts in the ovary tends to lead to menstrual irregularities, toward menorrhagia, and to be frequently associated with abdominal pains of crampy type as well as with dysmenorrhea. No significant endocrine therapy seems available.—E. L. S.

Treatment of gonorrheal vaginitis in childhood with estrogenic substances. Limper, M. A., and E. E. Hieronymus, J. Pediat. 9: 241. 1936.

In four cases of gonorrheal vaginitis treated with an ethylene glycal solution of amniotin, there were 2 permanent cures and 2 recurrences. With oily solutions of amniotin or of progynon-B administered intramuscularly twice a week, conversion from the infantile type of vaginal mucosa was observed and cures obtained in all but 2 cases. One patient with folliculin menformon daily for 60 days failed to respond to treatment. No harmful effects as a result of injections were observed.—M. B. G.

The effects of progestin on afterpains. Lubin, S., and F. J. Clarke, Am. J. Obst. & Gynec. 32: 134. 1936.

This study is of 55 multiparas, treated with 1 Rb.U. progestin intramuscularly. Beginning with the fifth day they gradually advanced the time of injection to 6 hours postpartum in the last 17 women. Complete relief was secured in 87% and in the 5 failures, 2 succeeded with a second dose, one had fibroids, another parametritis, and one was unexplained. The puerperium was otherwise unaffected.—E. L. S.

Reactions of mammary glands of normal and hypophysectomized male guinea pigs to female sex hormone. Lyons, W. R., and R. I. Pencharz, Proc. Soc. Exper. Biol. & Med. 33: 589. 1936.

The injection of large doses of estrin over a 1-month period into hypophysectomized male guinea pigs produced a nipple growth equal to that produced by the same dosage in normal male guinea pigs. By continuing the estrin injections at reduced levels, lactation was stimulated in the intact males, but not in the hypophysectomized males. The subsequent histological study of the mammary glands of the operated animals revealed very little alveolar development as compared to that found in the injected, normal males. It is inferred that the presence of the pituitary is necessary for the functional growth of the mammary gland.—W. H. F.

Intravaginal assay of urinary estrin. Lyons, W. R., and H. J. Templeton, Proc. Soc. Exper. Biol. & Med. 33: 587. 1936.

The urines of normal, cyclic, adult women were assayed for estrin content by introducing graded amounts of alcoholic extracts of such urines into the vagina of the castrate rats. The material was administered 2 times with a 24 hour interval, and the unit taken as the smallest amount required to produce cornification in 2 of 3 rats 24 hours after the last dose. Amounts of the extract representing 0.1 to 0.8 cc. of the original urine were found to be effective. (Cornification can be produced by the vaginal application of 1/200 of a R.U. of progynon.)—M. H. F.

The response of the mammary gland to prolonged stimulation with ovarian hormones. Macdonald, I. G., Surg., Gynec. & Obst. 63: 138. 1936.

Rabbits, mainly adult virgin females of various breeds were used in these experiments. Control specimens of mammary gland were excised in each animal before castration was done, or treatment begun. Castration was done by wide excision about the ovaries, and including portion of the oviducts. Estrin was generally supplied in the form of theelin and progynon B. Corpus luteum extract was tested for physiologic activity by the progestational effects produced. All hormone injections were given intravenously. The mammary glands were fixed in Bouins fluid, stained in hemalum, decolorized in acid alcohol, and used as whole mounts in balsam. The continuous administration of estrin in castrated rabbits produced at first a generalized overgrowth and ramification of the ductal system of the mammary gland with practically no acinar growth. Carried further, 100 days, little gross changes occur, but atypical histologic variations appear in the ductal epithelium which often verge on the border line of neoplastic changes. When such a stimulation is prolonged 6 months more, both the gross ductal growth and microscopic epithelial hyperplasia regress to a nearly resting state. Ductal widening is not prominent under estrin stimulation. In rabbits the response of the ductal system of the breast and its epithelium to continuous estrin stimulation is confined to certain limits past which a refractory state is set up. Estrin and corpus luteum extract in combination produce both ductal and acinar overgrowth, early secretion occurs and the ducts become distended. Dilatation of the ducts is probably mechanical from distension of secretion and is not a specific effect of estrin. On the basis of his experimental evidence the author is of the opinion that estrin and progestin do not produce specific pathological lesions, cystic disease and adenosis.—L. B. S.

The etiology of amenorrhoea with special reference to treatment. MacGregor, T. N., Edinburgh M. J. **43:** 1936. (Transactions Edinburgh Obstetrical Society 153.)

The author reviews our present knowledge of the endocrine factors in amenorrhoea. He insists that properly controlled estimations of female sex hormone and gonadotropic hormone are essential. He summarizes as follows: 71 cases of amenorrhoea have been studied. The underlying factors have been so divergent as to indicate that no one form of therapy meets each case. After excluding systemic disturbances, the therapy to be exhibited depends largely on repeated urine analysis correlated to the uterine findings. Treatment along endocrine lines will be more successful when more potent anterior pituitary preparations are available.—J. C. D.

The effect of progestin on the in vitro response of the rabbit's uterus to pituitrin. Makepeace, A. W., G. W. Corner and W. M. Allen, Am. J. Physiol. **115:** 736. 1936.

On the basis of reports that 2 active principles appear to exist in crude progestin, 1 responsible for progestational uterine changes and 1 concerned with desensitization of the uterus to pituitrin as tested in vitro, and in view of the fact that under in vivo conditions progesterone alone possesses the 2 properties, the authors designed experiments to determine whether progesterone might be capable of displaying both functions when tested in vitro. Sexually mature rabbits were prepared by injections of crude progestin in varying amounts over 48 hours, with autopsy 14 hours after the last injection, studies then being made of one cornu in a suitable physiological bath. It was found that 12.5 I.U. of pituitrin was ineffective for the most part in overcoming the inhibition produced, the exceptions occurring largely in animals which had been castrated 14-18 hours post coitum and then given progestin. Lack of consistent inhibition led to study of an additional series in which sexually mature rabbits, castrated at the same time interval after mating, were injected 4 times at 12 hour intervals with varying amounts of progestin (0.1 Rb. U. to 12 Rb. U.) followed after 14 hours by 1 I.U. of pituitrin. In general, as the amount of progestin administered was increased the number of animals showing complete or almost complete inhibition increased. A longer injection period gave more consistent results; 1 Rb. U. per day of impure extract or 1 mg. per day of B progesterone induced complete inhibition in every instance. Smaller dosages evoked variable results. Moreover, synthetic progesterone, prepared from stigmasterol, was found to possess the same capacity for inhibiting pituitrin action. It appears, therefore, that the pure hormone, progesterone, both natural and synthetic, under suitable conditions induces desensitization of the uterus.—H. O. Haterius.

Vitamin E and hormones. II. Restoration of fertility in female rats in vitamin E deficiency by grafting ovaries from normal rats. III. Restoration of fertility by administration of whole placenta extract (Vitamina E ed ormoni. II. Possibilità di ristabilire la fecondità in ratti femmine in istato di avitaminosi E mediante innesti di ovaia di ratti normali. III. Possibilità d ristabilire la fecondità in ratti femmine in stato di avitaminosi E mediante somministrazione di estratto totale di placenta). Marchesi, F., Sperimentale, Arch. di biol. **89:** 617; 622. 1935.

Rats rendered infertile by vitamin E deficiency returned to partial fecundity after receiving implantations of healthy ovaries. Injection of placental extract was superior by producing a normal fecundity. Less effective were anterior pituitary and pregnancy urine extracts. Lactation was missing but could be induced by feeding of liver.—A. E. M.

The restraining effect of follicular hormone on milk secretion in the puerperium. (Die hemmende Wirkung des Follikelhormons auf de Milchsekretion der Wöchnerin.) Mayor, J. M., Zentralbl. f. Gynäk. **60:** 2379. 1936.

Menformon and Progynon-B in oil, were injected intramuscularly, beginning within 24 hours after delivery of the placenta, in women in whom lactation was not desired. In 14 cases, the use of 200 to 1,000 mouse units daily for 6 to 8 days was without effect, confirming other reports. In 18 others the use of 10,000 units for 6 to 8 days was effective in a few cases, but only partially helpful. In the next 15 cases 250,000 units of Progynon-B were given in the first 10 hours, and 12 were completely inhibited. In the other 3 there were disturbing factors, and following a second injection of 200,000 units control was satisfactory.—E. L. S.

Progestin content of human placenta. McGinty, D. A., N. B. McCullough and J. G. Wolter, Proc. Soc. Exper. Biol. & Med. **34:** 176. 1936.

By the Corner-Allen method of extraction and the Allen and Meyer method of purification, it was possible to extract from as little as 150 gm. of human placental tissue enough

activity to produce a 2 to 3 plus reaction employing the McPhail technique. Even with the use of as much the equivalent of 300 gm. of tissue, it was not possible to secure more than a 3 plus reaction. This is attributed to estrin contamination.—M. H. F.

The amount of estrin in the blood and urine of pregnant women (Untersuchungen über die Östrinmenge in Blut und Harn bei Gebärenden). Moller-Christensen, E. and K. Pedersen-Bjergaard, Acta obst. et gynec. Scandinav. **16:** 142. 1936.

The correlation of primary uterine inertia and estrin production in pregnant women was investigated by the assay of both blood and urine in 22 and urine alone in 53 pregnant women. Primary inertia was present in 23 of the women studied. The estrin content of the urine in pregnancy varied from 1,000 to 100,000 M.U. per liter. The relation between the estrin in the blood and urine varied from 1-3 and 1-75. The authors concluded that primary uterine inertia could not be explained by a deficiency of estrin.—M. R. White.

The histology of the newborn and prepuberal prostate gland. Moore, R. A., Anat. Rec. **66:** 1. 1936.

The prostates from fifty foetuses and infants varying in age from 4 months gestation to puberty have been studied by the step section method. From its first appearance to the eighth month of gestation there is a gradual growth of the prostate which becomes accelerated in the ninth month. For 1 week after birth (reaching a peak at 4 to 7 days) there is morphological evidence of stimulation of the prostate to growth and secretion, which gradually subsides. The premature infant does not show this neonatal change. From this period to 10 years of age there is progressive growth with no secretory activity. From 10 years to puberty there is more rapid growth with slight secretion. At puberty the prostate reaches the full adult appearance.—Author's Abst., Wistar Bib. Service.

Evolution and involution of the prostate gland. Moore, R. A., Am. J. Path. **12:** 599. 1936.

This study is based on 678 prostates secured from consecutive autopsies at a hospital in Vienna. The glands were fixed in 10% formalin and stained by various methods. It is concluded that at puberty there is a rapid maturation of the prostate, probably due to an internal secretion of the testis which is activated by the pituitary gland. The mature postpuberal prostate is maintained as a uniform structure for about 25 years, except for occasional instances of pathological involution dependent on systemic disease. During the 5th decade of life involution begins and continues into the 8th decade. Evidence is cited to show that this involution is the result of a decrease and cessation of the same internal secretion which appeared at puberty.—W. J. A.

Depilatory action of a folliculin salve applied to the face of a woman with hypertrichosis (Action dépilatoire d'une pommade a la folliculine appliquée sur le visage d'une femme atteinte d'hypertrichose). Mussio-Fournier, J. C., A. Bertolini, J. Morato-Manaro and W. Bruño, Bull. Acad. de méd. **113:** 352. 1935.

The hypertrichosis developed concomitantly to menstrual disorders (menorrhagias). No adrenal involvement was noted. A folliculin salve on ammonium stearate basis was applied to the skin with excellent results. The effect was restricted to the site of application.—A. E. M.

A brief epitome of gynecologic endocrinology and organotherapy. Novak, E., Am. J. Obst. & Gynec. **32:** 887. 1936.

This is a presentation of the current understanding of the interrelations involved, with therapeutic indications. It is concise, accurate, and as it is a review cannot be presented in abstract form. There has not appeared to date a rival for this article.—E. L. S.

A histological study in twenty-four cases of retained testes in the adult. Pace, J. M., and H. Cabot, Surg. Gynec. & Obst. **63:** 16. 1936.

Twenty-four cases of retained testes from the second through the seventh decade are presented with detailed histological study. Three were found which showed evidence of cancer; 2 of these were in the third decade and showed a lesion described as adenocarcinoma. The third was in the seventh decade, showing a lesion which was controversial, most pathologists regarding the cells as showing abnormal hyperplasia. The findings suggest that mal-position adds to the likelihood of malignant changes.—L. B. S.

Effects of ovarian transplants upon the development and maintenance of the seminal vesicle and prostate gland of the albino rat. Pfeiffer, C. A., Anat. Rec. **65:** 213. 1936.

A limited amount of development proceeds in the seminal vesicles and prostate of the male rat castrated at birth. This includes some formation of smooth muscle which is hypertrophied by the estrin produced by an ovarian graft. Otherwise the normal differentiation under the influence of the ovary does not proceed any further than in the castrate. After considerable time the epithelium may be changed from the low castrate type to a stratified squamous epithelium. This may in the coagulation gland become keratinized. The effects differ in the various glands by degree only. The smooth muscle hypertrophy is greater in the seminal vesicle while the metaplasia of the epithelium is greater in the coagulation gland. The age at which either castration or ovarian grafting is performed is varied from birth up to and including the adult. The results seem to be dependent upon the amount of differentiation obtained before castration rather than the time of grafting. Male hormone is necessary for normal male differentiation while estrin tends to differentiate toward femaleness. A possible explanation of the above facts, based upon embryology, is discussed.—Author's Abst., Wistar Bib. Service.

Local uterine growth in untreated ovariectomized rabbits. Reynolds, S. R. N., and S. Kaminester, Proc. Soc. Exper. Biol. & Med. **34:** 69. 1936.

In castrate female rabbits chronic local uterine distention resulted in growth of muscle and endometrium, in contrast with the progressive atrophy of the undistended uterine horn.—M. H. F.

The age of sexual maturity in 250 albino female rats (Mus norvegicus albicus, Wistar Strain). Rigdon, R. H., J. Lab. & Clin. Med. **21:** 1182. 1936.

The author studied the age of the opening of the vagina in 250 rats of the Wistar strain. He found a variation between 28 and 59 days with an average of 42.1 days of age. The age of sexual maturity was not influenced by weight, age of weaning, presence of males, or season of year.—F. A.

Light regulation of sexual activity in the male sparrow. Riley, G. M., Proc. Soc. Exper. Biol. & Med. **34:** 331. 1936.

A group of male sparrows exposed to increasing light ration from a 100 watt bulb gave evidence of definite testicular stimulation and male hormone production (bill reaction), when the exposure to the light was initiated in November. These results are in accord with those of Bissonnette, and other workers. Yet, the same treatment given to a group of mature and immature male sparrows in September, resulted in definite stimulation of the testes of the immature birds, but had little effect on the testes of the mature birds. It is concluded that in the adult male sparrow there is an intrinsic sexual rhythm, which becomes synchronized with the seasons through environmental factors.—M. H. F.

Hormones and pregnancy. Robson, J. M., Brit. Med. J. **1:** 1033, 1936.

This is a comprehensive review article in which the author presents the current conceptions and his own interpretation of the physiological activity of anterior hypophyseal and gonadal hormones in normal and pregnancy states.—H. B. Friedgood.

The rôle of the luteal hormone in the maintenance of gestation. Robson, J. M., Edinburgh M. J. **43:** 395. 1936.

This summarizes our present knowledge of the hormonal control of gestation. Special stress is laid on the differences found between various kinds of animals and man.—J. C. D.

The action of oestrin on the bitch. Robson, J. M., and W. R. Henderson, Proc. Roy Soc., London, s.B., **120:** 1. 1936.

Four hypophysectomized bitches were treated with oestrone or oestriol in doses of approximately 0.5 mg. daily for 10-20 day periods. This produced willingness to mate, swelling of the vulva, hypertrophy of endometrium and myometrium and uterine bleeding not only during but also after the injections. Confirmatory results were obtained with ovariectomized bitches. Experiments on strips of uterine muscle removed from time to time showed that the in vitro reactivity to pituitary oxytocin remains relatively high after ovariectomy or hypophysectomy, and is not further heightened by oestrin treatment.—D. L. T.

Sex hormones (Sexualhormone, XIII). Ruzicka, L., and H. B. Rosenberg, Helv. Chim. Acta **19:** 357. 1936.

The reduction of the 17-keto group in compounds of the male hormone series in the usual way with alcoholic sodium hydroxide yields secondary alcohols, such as andradiol, in which the hydroxyl group is in trans-position to the 13-methyl group (as in testosterone). Compounds with the 17-cis-hydroxy configuration are obtained if the reduction is carried out with the Grignard reagents, ethyl or propyl magnesium iodide. Isomers of the known andradiol and androstene-diol have been prepared in this way for the first time; biological activity does not appear to be greatly affected by this change in configuration. With methyl magnesium iodide, 17-methyl-3, 17-diols are obtained as principal products; 17-methyl-testosterone is the most potent known member of the whole series, in its action on the seminal vesicles of immature male rats.—D. L. T.

Testicular hormone in the blood of the spermatic vein (Sur la présence de l'hormone testiculaire dans le sang de la veine spermatique). Sacchi U., and A. Fraschini, Arch. ital. de biol. **94:** 14. 1935.

The hormone was assayed by the cock comb method and by measuring the development of secondary sex organs in castrate male rats. The blood of the spermatic vein contained more testicular hormone than the blood of the general circulation.—A. E. Meyer.

Estrogenic activity of the blood (Actividad estrógena de la sangre). Schlossberg, T., and C. A. Durruty, Rev. Soc. argent. de biol. **12:** 77. 1936.

Alcoholic extracts of blood and serum (1 to 5 cc.) of normal women produced estrus in spayed rats. The blood from ovariectomized and menopausic women and from men also had an oestrogenic activity in approximately the same amount as that from normal women. Frank's method of extraction does not permit the recovery of all the estrogenic substance; from the residue additional amounts were obtained by further extraction with boiling alcohol. It is deduced that the nature of the estrogenic substance must be considered as unknown and should not be identified with oestrone until further data are collected, since the estrogenic activity of blood bears no relation with the different sexual functional conditions.—J. T. L.

Changes in the incisor of the thirteen-lined ground squirrel (Citellus tridecemlineatus) following bilateral gonadectomy. Schour, I., Anat. Rec. **65:** 177. 1936.

The effect of gonadectomy on the incisor has been studied in 26 bilaterally gonadectomized thirteen-lined ground squirrels (25 to 339 days after operation) and 33 controls in respect to radiographic and microscopic alterations. Roentgenograms showed an abnormal extent of radiolucent dentin, in 54% of the experimental animals. The histologic changes consisted of an abnormal persistence of the organic enamel matrix in the middle or distal third of the incisor, of an abnormal width of the osteoid border in the alveolar bone, and chiefly of alterations in the dentin. The latter showed 3 distinct zones which were disturbed in calcification and which disregarded the incremental pattern. In severe cases, the formation of dentin was also disturbed. The histologic changes are essentially of the nature of calcification disturbances but are unlike those seen in the incisors of rats which were hypophysectomized, parathyroidectomized or adrenalectomized or fed a rachitogenic diet. The material presented in this paper throws interesting lights on various phases of the calcification processes of teeth.—Author's Abst., Wistar Bib. Service.

Effect of combined administration of oestrone and progesterone in adult ovariectomized rats. Selye, H., J. S. L. Browne and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **34:** 198. 1936.

The simultaneous injection of 400 gamma of progesterone and 30 gamma of oestrone daily for 20 days produced only dioestrus smears in 5 of the 6 animals observed. Autopsy at the 20th day revealed marked vaginal mucification, and in 4 of the 6 cases the uterus showed the second stage of progestational proliferation as seen in late pregnancy. It is concluded that the vaginal effects and the uterine changes seen in late pregnancy are due to the combined action of estrin and corpus luteum hormone.—M. H. F.

Effect of testosterone on the mammary gland. Selye, H., C. S. McEuen and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **34:** 201. 1936.

The injection of 200 gamma of testosterone daily for 23 days produced slight mammary gland development and marked milk secretion in immature male and female rats, normal and castrate.—M. H. F.

The effect of ovarian hormone on the basal metabolism of experimental hyperthyroid rats. Sherwood, T. C., and L. M. Bowers, Am. J. Physiol. **115:** 645. 1936.

The effect of the injection of amniotin (Squibb) in oil, and of theelin (Parke Davis) in water on the basal metabolism of normal and experimental hyperthyroid albino rats was investigated. Theelin reduced by 10% to 24% the metabolism of 5 of 7 normals. Amniotin reduced the metabolism of 4 normals 28% to 54%. Injections of 100 rat units of amniotin per day for 3 days caused the basal metabolism of 9 experimental hyperthyroid animals to return to normal in from 3 to 5 days. while the metabolism of 6 control animals returned to normal in 8 to 10 days. The ovaries produce an effect upon thyroid function in the control of basal metabolism.—F. A. Hitchcock.

An interstitial-cell tumor of the testis with hypergenitalism in a child of 5 years. Stewart, C. A., E. T. Bell and A. B. Roehlke, Am. J. Cancer **26:** 144. 1936.

An interstitial-cell tumor of the right testis with hypergenitalism is reported in a boy 5 years of age. This is the third case in the literature. Pubic hair was present, the penis greatly enlarged, and the testis containing the tumor had developed almost to the point of formation of spermatozoa. Following removal of the affected testis the pubic hair mostly disappeared and the penis became smaller. This case adds further evidence that the secretion of the interstitial cells is important in the development of the secondary sexual characteristics of the male.—R. G. H.

Management of secondary amenorrhea of functional origin. Tamis, A. B., Am. J. Obst. & Gynec. **32:** 845. 1936.

Together with reports on 25 cases there is discussion of theoretical relations. Urine was tested for estrogenic and gonadotropic substances by methods recognized to give less than 100% yields. The cases are empirically grouped into a series of types. Best results were obtained in cases with hypothyroidism, or with use of thyroid in other cases. Some benefit was seen from x-ray therapy (recognized to be destructive) and from use of amniotin and follutin.—E. L. S.

Demonstration of small amounts of gonadotropic hormones in the urine, especially in normal individuals (Nachweis kleiner Mengen gonadotropen Hormons in Harn besonders von Normalen). Thomsen, O., and K. Pedersen-Bjergaard, Ztschr. f. Geburtsh. und Gynäk. **112:** 202. 1936.

A method was described for demonstrating small amounts of gonadotropic hormone in the urine of normal individuals by precipitation with freshly prepared 10% tannic acid. The precipitate was taken up in water and brought to a pH 9.5 by additions of a boric buffer. A standard strain of rats was used; the test animals were from 30-35 days old and did not weigh more than 35 grams. The different stages in the reactions in the vagina and uterus were illustrated. As little as 1-1/5 R.U. was demonstrated in a 24 hour specimen.—M. R. White.

Cyclical changes in the human vaginal mucosa. Traut, H. F., W. Block and A. Kuder, Surg., Gynec. & Obst. **63:** 7. 1936.

This study is based upon specimens taken from 29 normally menstruating women who had no gynecological or endocrine condition which might vitiate the observations. In 12 of these women it was possible to secure 4 or more vaginal biopsies at weekly intervals so that there is represented in them a complete menstrual cycle. From the remaining 17, either a lesser number of specimens were secured or intervals longer than a week elapsed between taking of biopsies. The conclusions were based principally on the former group. All biopsies were taken at the same level in the middle of the upper third of the vagina. It was found that there is a cyclic change in the vaginal mucosa correlated with phases of the sex-cycle. The cellular response is characterized by a proliferation on part of the stratum germinativum with increased number of young epithelial cells in the basophilic zone. This response is associated with occasional mitosis and a very definite leucocytosis and hyperemia. The proliferative phase appears in the premenstruum, lasts 6-7 days and is either completed premenstrually or extends into the menstrual phase and occasionally to the postmenstruum. It has been observed between the 7th and 21st days of the cycle. Between proliferative phases the epithelium is quiescent. Such alteration between proliferative and inactive phases has not been demonstrated in pregnancy, which seems to indicate that the rhythm of the vagina is related to the ovarian cycle.—L. B. S.

Observations on the biochemistry of the genital tract of the female macaque particularly during the menstrual cycle. van Dyke, H. B., and G. Ch'en, Am. J. Anat. **58:** 473. 1936.

Total solids, ash, glycogen and total nitrogen were determined in the mucous membrane and the musculature of the corpus uteri, cervix uteri and vagina of the monkey during the

stages of proliferation (follicle ripening), secretion (corpus luteum), and menstruation. The genital tracts of ovariectomized animals, most of which received injections of follicular hormone (theelin), were similarly analyzed. The glycogen of the vaginal mucosa, which is normally present in high concentration and rapidly disappears after ovariectomy, was found to be very stable in comparison with the glycogen of the liver and of striated muscle. Observations on the relationship between the amount of glycogen in the vaginal mucosa and the hydrogen-ion concentration of the vaginal fluid were also made.—Authors' Abst., Wistar Bib. Service.

Action of oestrin on the vagina during lactation. Votquenne, M., Proc. Soc. Exper. Biol. & Med. **34:** 207. 1936.

The injection of 0.2 R.U. daily during the first 4 days of lactation, or the injection of 0.75 R.U. daily from days 5-9 of lactation, resulted in the typical histological picture of estrus in the vagina, although the vaginal smears remained 'falsely negative.' The latter is attributed to the presence of large numbers of leucocytes even in the presence of complete keratinisation. These results are not in accord with those of Selye, who used much larger doses of estrin which resulted in mucification. The antiestral activity of the corpora lutea of lactation can be observed only when the doses of estrin injected are less than 1 R.U.—M. H. F.

The effect of estrin on the prostate gland of the albino rat and mouse. Weller, D., M. D. Overholser and W. O. Nelson, Anat. Rec. **65:** 149. 1936.

Daily injections of 40 R.U. of estrin (Amniotin) were given to 6 normal and 6 castrate adult male rats over 39- and 67-day periods. In 6 animals 50 R.U. of Antuitrin-S were also injected daily during the last 18 days of estrin injections. No metaplasia of the prostatic epithelium or bladder dilatation occurred in any animals. Eight normal and 9 castrate adult mice received 40 R.U. of estrin daily over periods ranging from 8 to 21 days. Bladder dilatation occurred in all but 3 of the animals. No histological study of the prostates was made but they were decreased in size as compared with controls. Eight normal and 9 castrate adult mice received 40 R.U. of estrin daily over 13- and 29-day periods. Bladder dilatation occurred in all animals. The prostates were decreased in size, and serial sections in 14 cases showed a metaplasia of the columnar epithelium into stratified squamous. This change was most pronounced in the anterior lobes, and more marked in castrates and in those receiving the longer injections. Four adult male mice received 400 R.U. of estrin in 15 days. Two received 500 R.U. of Antuitrin-S and 2 received 25 bird units of male hormone in addition to the estrin. Bladder dilatation occurred in all cases but there was no metaplasia of prostatic epithelium.—Authors' Abst., Wistar Bib. Service.

Effect of light on reproductive cycle of Peromyscus leucopus noveboracensis (white-footed mouse) Whitaker, W. L., Proc. Soc. Exper. Biol. & Med. **34:** 329. 1936.

Increasing daily light rations from a sunlamp stimulated a group of 35 white-footed field mice to sexual activity 6 to 8 weeks before such activity appeared in a similar group which did not receive the extra light, but which did receive the heat rays from an infra-red lamp.—M. H. F.

An unusual double human pregnancy with a single corpus luteum. Wieman, H. L., and C. K. Weichert, Anat. Rec. **65:** 201. 1936.

An account of conditions found in a double human pregnancy of about 2 months' duration, the outstanding features of which are, 1) a single corpus luteum of pregnancy; 2) two male embryos, each enclosed in a separate amnion and chorion, and 3) widely separated implantation sites. The tentative conclusion is reached that the embryos developed from 2 eggs produced simultaneously by a single ovarian cycle.—Authors' Abst., Wistar Bib. Service.

Some points in the treatment of endometrial hyperplasia by progesterone therapy. Wilson, K. M., and C. A. Elden, Am. J. Obst. & Gynec. **32:** 194. 1936.

Case histories of 5 women with vaginal bleeding and demonstrated endometrial hyperplasia are given. The use of doses of from 1/25 to 15/25 Rb.U. total were adequate to interrupt the bleeding. Frequently the difficulty recurred, but was again brought under control by the same therapy. No tissues were obtained to demonstrate whether or not a progestational change occurred in the endometrium.—E. L. S.

Treatment of gonorrheal vaginitis in childhood with the ovarian follicular hormone. Series of cases in which treatment was unsuccessful. Witherspoon, J. T., Am. J. Dis. Child. **50:** 912. 1935

Ten children with gonorrheal vaginitis were unsuccessfully treated with ovarian follicular hormone. The vaginal discharge and gram negative intracellular diplococci in vaginal smears

persisted. Several disadvantages are pointed out: the harmful effects on the immature ovary; cervical infection in addition to vaginal as a focus for continuing the disease; the occasional secondary effects as enlargement of the breasts, vulval hyperemia and hypertrophy; and the high cost of the therapy.—M. B. G.

Comparison of the Corner-Allen and Clauberg tests for assay of progestin. Young, L. E., Proc. Soc. Exper. Biol. & Med. **34:** 96. 1936.

Progestin was assayed on mature (Corner-Allen) and immature rabbits primed with estrin (Clauberg). The Clauberg unit was found to be equal to about ½ Corner-Allen unit. It was also found that the immature uterus was much more variable in response than the mature uterus, so that to secure the same accuracy more immature animals must be employed than in an assay on mature animals.—M. H. F.

Inhibition and induction of uterine bleeding by means of oestrone. Zuckerman S., Lancet **231:** 9. 1936.

The author in presenting this paper purposes to examine the facts bearing on 2 points; 1) that continuous injection of hormone inhibits menstrual bleeding and, 2) that uterine bleeding occurs even in ovariectomized human beings and monkeys during prolonged oestrin treatment. In attempt to test the oestrin withdrawal theory, oestrone was administered daily in doses varying between 250-1000 R.U., between the 18th and 29th days, that is, at mid-point of the cycle after ovulation had presumably occurred. In the regular menstruating monkey (Cercocebus) this treatment did not disturb the menstrual rhythm. If, however, the injections were started on the 12th day, before ovulation had occurred, and before formation of the corpus luteum, the subsequent menstruation was inhibited. The fact that uterine bleeding occurs during continuous oestrone injections only when the amount given daily is a threshold or subthreshold dose is brought out in 2 experiments. One monkey never bled in the course of 220 days during which it was given 1000 I.U. of oestrone daily. Another on 100 I.U. daily bled between the 48th and 52nd days. The available evidence does not suggest that oestrin actively stimulates uterine bleeding, but still favors the view that bleeding is an event which marks the cessation of the hormone's action.—L. B. S.

Influence of oestrogens on the prostate gland. Zuckerman, S., Nature **137:** 1032. 1936.

Benign spontaneous enlargement of the prostate is commonly seen in aged dogs; a case is briefly described in which the epithelial changes seen in prostates experimentally enlarged by estrin-administration have arisen spontaneously. It is likely that estrin is the usual cause of prostatic enlargement but is not commonly present in sufficient amount to produce the characteristic histological changes.—D. L. T.

HYPOPHYSIS

A comparative study of the thyroid glands of hypophysectomized newts after treatment with anterior pituitary, thyroid and iodine. Adams, A. Elizabeth, and Beatrice Gray, Ant. Rec. **65:** 69. 1936.

Newts hypophysectomized for 6 to 12 weeks were treated with anterior pituitary grafts or injections of thyroid extract or iodine. Muscle transplants or salt solution injections served as controls. The cornified layers of skin that accumulate after pituitary removal can be caused to be shed by any of the first three treatments, but not by the last two. Following the molting, the thyroids were fixed and studied. Anterior pituitary activates the thyroid gland, but thyroxin and iodine administration leave it in the inactive condition characteristic of the untreated hypophysectomized newt or of those receiving muscle or salt solution treatment, so that their ability to set in motion the molting mechanism must be a direct one and not mediated by the thyroid. The lack of response of the thyroid of the hypophysectomized newt to iodine points to the control that the anterior pituitary exercises on the thyroid's ability to utilize iodine, since in the normal animal iodine treatment activates the thyroid.—Authors' Abst., Wistar Bib. Service.

The blood-sugar action of an anterior lobe pituitary fraction after removal of adrenals (Über die Blutzuckerwirkung von Hypophysenvorderlappen-Fraktionen nach Ausschaltung der Nebennieren). Anselmino, K. J., and F. Hoffman, Arch. f. exper. Path. u. Pharmakol. **181:** 675. 1936.

The authors found that the pancreatrope factor, (tested for activity by histological examination of the pancreas) has practically no effect on the blood sugar level of the intact dog. In

animals with ligated adrenal veins the blood sugar may be lowered as much as 40-50 mg. % in 7 hours after a subcutaneous injection of the pancreatrope fraction. The pancreatrope factor was destroyed by heating the solution on a water bath for 15 minutes. If the ultrafiltrate remaining after such treatment is injected into the intact animal there is an increase of the blood sugar level. Ligation of the adrenal veins has no effect on the action of this latter substance. This blood sugar increasing factor was present in the pituitary extract at a pH of 5.4, but its action did not become apparent until the pancreatrope factor was destroyed. Blood sugar increases as high as 20 mg. % were produced within 5-7 hours after injection. The authors consider this blood sugar increasing factor distinct from the contra-insular factor.—R. Cleveland

Preparation of the carbohydrate metabolism hormone of the anterior pituitary (Zur Darstellung des Kohlehydratstoffwechselhormons des Hypophysenvorderlappens). Anselmino, K., and F. Hoffmann, Endokrinologie **17:** 289. 1936.

Crude extracts of beef anterior pituitary are brought to pH 5.2-5.4 by the addition of N/2 acetate buffer and subjected to ultrafiltration. At this reaction most of the carbohydrate metabolism hormone passes through and the fat metabolism hormone is held back. Urine of diabetics is precipitated by the addition of 5 vols. of 99% alcohol. The precipitate is washed many times with absolute alcohol and ether. The yellowish-white powder is dissolved in water and ultra-filtered at pH 5.2-5.4. In order to obtain the hormone from the blood serum of the normal animal it is necessary to administer dextrose by mouth or intravenously.—W. J. A.

Antagonistic action of fat metabolism hormone and of carbohydrate metabolism hormone of anterior lobe hypophysis by the hormone of the adrenal cortex (Über die antagonistische Beeinflussung der Wirkung des Fettstoffwechselhormons und des Kohlehydratstoffwechsel hormones des Hypophysenvorderlappens durch das Hormen der Nebennierenrinde). Anselmino, K. J., F. Hoffmann and E. Rhoden, Arch. f. exper. Path. u. Pharmakol. **181:** 325. 1936.

The normal blood acetone and liver glycogen were determined in normal rats in mg. % and gm. % respectively. After administration of a crude pituitary extract containing both the fat-metabolizing and carbohydrate-metabolizing factors the blood acetone and liver glycogen were again determined. The blood acetone was increased from values of 4-6 mg.% in the controls to 10-13 mg.% in the experimental animals, while the liver glycogen was decreased from values of 4.5 to 4.9 gm.% in the controls to values of 1.0 to 1.9 gm.% in the experimentals. When 2 cc. adrenal cortical hormone was given simultaneously with the crude extract the blood acetone values ranged from 4.4-7 mg.% with an average of 5.5; the liver glycogen values lay between 3-4 gm.% with an average of 3.9. With the purified extracts of the pituitary and the simultaneous administration of the adrenal cortical hormone, the same antagonism of the cortical hormone action to that of the fat metabolizing and glycogenolytic factor of the carbohydrate metabolizing hormones of the pituitary were observed.—R. Cleveland.

The desposition of fat in the liver after treatment with the fat metabolism hormone of the anterior lobe of the hypophysis (Ueber Leberverfettung durch Behandlung mit dem Fettstoffwechselhormon des Hypophysenvorderlappens). Anselmino, K. J., F. Hoffmann and E. Rhoden, Arch. f. d. ges. Physiol. **237:** 515. 1936.

The daily injection of an alkaline extract of beef pituitary glands for 3 days increased the fat content of the rat's liver 50%. The active principle was ultrafilterable at pH 9.4.—A. G.

The relation of the hypophysis to the growth of malignant tumors. III. The effect of hypophysectomy on autogenous tumors. Ball, H. A., and L. T. Samuels, Am. J. Cancer **26:** 547. 1936.

The weights of dibenzanthracene tumors were estimated by measurements in 7 hypophysectomized and 7 control rats. The average weight of tumor growth over a period averaging 34.3 days in each group was 0.33 gm. daily for the hypophysectomized and 2.45 gm. for the controls. It is concluded that the cause of the difference was deprivation of pituitary hormone.—R. G. H.

Glucose absorption and glycogen formation in the hypophysectomized rat. Bennett, L. L., Proc. Soc. Exper. Biol. & Med. **34:** 277. 1936.

Using the methods of Cori, it was found that hypophysectomized rats absorb less glucose than normals (confirming Phillips and Robb), and that with the same amount of glucose by

mouth, the operated animals deposited less liver glycogen (5.9 mg. per 100 gm. of body weight; 2.4% of the absorbed glucose) than the normal animals (60 mg. of new liver glycogen; or, 14.5% of the absorbed glucose.) Calculations from muscle glycogen determinations showed a diminished muscle glycogen deposit from the glucose absorbed by the operated animals.—M. H. F.

Diabetes insipidus: the site of the formation of the anti-diuretic hormone. Biggart, J. H., Edinburgh M. J. **43:** 417. 1936.

Two cases are reported in which damage was localized in the posterior lobe and supra-optic nuclei. The appearance of diabetes insipidus associated with such circumscribed lesions gives support to the view that in man posterior lobe basophiles are not analogous to the pars intermedia of animals; that they do not secrete the anti-diuretic factor; and that they are not innervated by the supra-optic hypophyseal nerve fibers. Secretion of the anti-diuretic hormone by the pars tuberalis or the posterior lobe would fit the picture presented in these cases.—J. C. D.

Observations on the origin of the horns of the roebuck. (Beobachtungen über die Entstehung der Perücke beim Rehbock). Blauel, G., Endokrinologie **17:** 369. 1936.

A roebuck which recently had shed it horns was injected with 7 doses of 50,000 M.U. each of progynon—a total of 350,000 U. After the first injection the velvet was visibly stimulated and after the third the horns became noticeable. The horns later went on to normal full development. The rôles of the hypophysis and of the testes are considered.—W. J. A.

Diencephalic regulation of glucemia (Sulla regolazione diencefalica della glicemia) Borhetti, U., Sperimentale, Arch. di biol. **89:** 560. 1935.

Introduction of oxygen, air and, to a less degree, water into the cerebral ventricles of the rabbit produces a marked hyperglucemia followed by a hypoglucemic phase.—A. E. M.

Relative gonadotropic augmentative action of plasma and formed elements from blood of cattle. Casida, L. E., Proc. Soc. Exper. Biol. & Med. **33:** 570. 1936.

The addition of beef plasma to pituitary extract did not noticeably augment the ovarian response elicited in the injected rats, whereas the addition of the formed elements from the same blood produced marked augmentation. The augmenting action of the formed elements was destroyed by incineration, but not by boiling.—M. H. F.

Hypophyseal findings in infantilism (Über den Hypophysenbefund bei Ateleiose). Ceranke, P., Wien. Arch. f. inn. Med. **29:** 151. 1936.

A case is described of hypophyseal infantilism in a 50-year-old woman, 129 cm. tall, without genital dystrophy and without cranial pressure symptoms. The anterior lobe of the hypophysis was unusually small and there was an absolute increase of principal cells over chromophils.—W. J. A.

The amount of thyroid-stimulating hormone in the anterior lobe of the pituitary of the thyroidectomized rabbit. Chen, G., and H. B. Van Dyke, Chinese J. Physiol. **10:** 285. 1936.

Young rabbits were thyroidectomized when 8 to 10 weeks old; of 39 animals, 31 had littermate controls. Operated and control animals were killed simultaneously 14 to 227 days after thyroidectomy when a careful search was made for remnants of thyroid tissue and the pituitary body was removed. The enlarged anterior lobe was then separated, weighed and a suspension prepared for injection into immature guinea pigs which were given 4 daily doses and killed on the 5th day. The total control dose of fresh anterior pituitary was usually 6.0 mg.; the amount from thyroidectomized rabbits was usually the same (for detecting a change in concentration) or proportional to anterior pituitary weight (for changes in total hormone content). Using guinea pig thyroid histologic and weight changes as criteria, it was found that the total amount of thyrotropic hormone in the anterior pituitary was increased in female rabbits thyroidectomized for 3 months or longer. Similarly operated male rabbits had larger anterior pituitaries, but no greater amount of thyrotropic hormone. Hypertrophied pituitaries contained more water than normal glands.—R. L. Z.

On the biological properties of mare gonadotropic hormone. Cole, H. H., Am. J. Anat. **59:** 299. 1936.

Ten of 12 25-day-old rats injected with 2 R.U., and 8 of 12 injected with 16 R.U. ovulated. Superovulation occurred in 2 rats of the latter group but superovulation could not be induced in the mature rabbit by increasing the dose above the minimum ovulating level. Ovulation occurs about 72 hours after the injection in the rat, ewe, cow and sow. In rats injected on the tenth day of age follicles are not brought to maturity. Large doses produce marked interstitial cell growth but only slight evidence of follicular growth. Counts of all normal follicles in every fourth section of ovaries of rats injected on the 25th day show that there is a slight decrease in the number of primordial follicles and an increase in the number of larger follicles aside from those containing definitive ova. The normality of the response in the 25-day-old rat is attested by the fact that they breed, carry young to term, and occasionally raise young to the time of weaning. The male rat shows a response with an amount very little larger than that necessary to produce a response in the female. After a single injection there is interstitial cell growth and tubular enlargement but after chronic treatment the effect is only apparent in the interstitial tissue of the testis and in the accessory organs.—Author's Abst., Wistar Bib. Service.

Quantitative study on the adrenals of hypophysectomized rats. Cutuly, E., Anat. Rec. **66:** 119. 1936.

The profound atrophy occurring 30 days after operation in the adrenal glands of 20 adult male and 24 adult female rats was found to be due, on the basis of statistical evidence, entirely to shrinkage of the cortex. There was proportionally more shrinkage in the adrenal cortex of females than of males, so that their weights tended to approach a common minimum. —Author's Abst., Wistar Bib. Service.

Action of anterior pituitary extracts upon acetone body content of the blood (Über die Wirkung von Hypophysenvorderlappenextrakten auf den Azetonkörpergehalt im Blute). Dingemanse, E., Endokrinologie **17:** 292. 1936.

In testing about 40 anterior pituitary extracts upon about 300 rats fed the same diet of white bread and milk, only a few preparations gave any clear action upon ketone bodies of the blood. No action at all could be detected when rabbits were used. Rats showed great individual variation in reaction, 50 mg. of a preparation being required to produce an increase of blood ketones in certain animals, which, in others, could be called forth by the administration of as little as 10 mg. The effect of fasting is very marked. After 6 hours normal rats show a decided increase of ketone bodies in the blood. Also the time of day makes great differences. The ratio of beta-oxybutyric acid to acetone plus acetoacetic (2-10) does not change after injection of active pituitary extracts; the same is true in fasting. It is concluded that a special hormone for the control of ketone bodies has not been definitely demonstrated.—W. J. A.

The vegetative nervous system and the functional activity of the hypophysis of amphibians (Le systeme nerveux vegetatif et l'activite fonctionnelle de l'hypophyse chez les amphibiens). Doubovik, I. A., Rev. franç. d'endocrinol. **14:** 151. 1936.

In immature female frogs stimulation of the anterior lobe with an electric current accelerated maturation of the testes; stimulation of the stellate and vagus ganglions elicited no change in the ovaries. Twenty-five frogs were used in each experiment as well as controls. The melanophoric reaction was tested in 30 immature female frogs and axolotls by stimulation of stellate and vagus ganglions, by extirpation of stellate ganglion and by bilateral vagotomy before and after hypophysectomy. Normal and hypophysectomized axolotls were further tested by skin transplants from one to the other. The transplants adopted the coloration of the new host after three months. The author concluded that the hormonal activity of the anterior lobe is independent of the nervous system and that the secretory activity of the posterior lobe is autonomic and is adapted by the vegetative nervous system.—W. L. Thomas, Jr.

The estrous cycle and weights of organs in relation to the hypophysis in the hairless rat. Emery, F. E., Am. J. Physiol. **111:** 392. 1935.

A hairless strain of rats has been studied in respect to the estrous cycles, weights of organs and pituitary-gonad relationship. The data from more than 200 hairless rats were compared to the half-breed hairless (heterozygous) and to normal albino rats. The spleen, pituitary,

thyroids and testes are similar in weight in albino, heterozygous and hairless males. The kidneys and adrenals are larger in heterozygous and hairless rats of both sexes. The ovaries and uterus are smaller in the hairless females than in heterozygous and albino females. The estrous cycles are prolonged in the diestrous phase, usually irregular and often absent. The hairless female may give birth to young but is unable to lactate. The gonadal stimulating hormone of the hypophysis is greatest in amount in hairless males and females, intermediate in the heterozygous and least in albino rats. After castration in hairless and heterozygous females the pituitary is quite inferior to the pituitaries of castrated albino rats as judged by the implant method of testing. A possible relationship between the hypophysis and the hairless condition is discussed.—Author's Abst.

The gonadotropic function of the anterior pituitary in the climacteric and the follicular hormone effects upon it. (Ueber die gonadotrope Funktion des Hypophysenvorderlappens im Klimakterium und deren Beeinflussung durch Follikelhormon.) Engelhart, E. and E. Tscherne. Zentralbl. f. Gynäk. **60:** 790. 1936.

Morning urine was concentrated by Zondek's method of alcohol precipitation and injected into 6-8 gm. mice in a series of 18 doses, followed by autopsy at 100 hours. Luteinizing hormone was not found before or after therapy, which employed progynon-B in oil, 60,000 to 100,000 mouse units. Results with 4 women of ages 60-65 show a definite diminution of the gonadotropic hormone excretion, which is temporary in 2 of 3 cases followed long enough to determine the time of action.—E. L. S.

Effect of feeding of thyroid or salt and of thyroidectomy on fluid exchange of cats with diabetes insipidus. Fisher, C., and W. R. Ingram, Arch. Int. Med. **58:** 117. 1936.

Cats with experimental diabetes insipidus due to bilateral interruption of the supra-optico-hypophyseal tracts respond to such diuretics as thyroid and salt with a greater than normal increase in water exchange. Also, cats with partial injury to the supra-optico-hypophyseal system in which permanent polyuria does not develop show similar abnormal increases in water exchange in response to these diuretics. The excessive diuresis is interpreted as indicating an absence of, or decrease in the amount of, antidiuretic substance in the body. The removal of the thyroid gland from 3 cats with permanent polyuria resulted in a ¼-½ diminuition of the water exchange. The subsequent feeding of thyroid to 2 of these animals brought on marked diuresis, which was followed by a return not to the preceding level but to a level more nearly corresponding to that prevailing before thyroidectomy. This new level persisted long after the effect of the thyroid had worn off. The evidence from these experiments does not support the theory that the diuretic effect of the anterior lobe of the pituitary gland is a specific effect mediated through the thyroid gland.—I. B.

Carbohydrate oxidation in hypophysectomized rats. Fisher, R. E., and R. I. Pencharz, Proc. Soc. Exper. Biol. & Med. **34:** 106. 1936.

Rats hypophysectomized 6-8 weeks previously, showed relatively high R.Q's, despite lowered B.M.R. The operated animals lost liver and muscle glycogen more rapidly than controls on a 24 hour fast. When glucose was fed to fasted rats previously on a high carbohydrate diet, the operated animals burned somewhat less than the controls, but in view of the markedly lowered B.M.R. the sugar burned represented a larger portion of the total energy. When the rats were fed on a high fat diet for 2 days prior to glucose administration, the glucose burned by the normal rats was less (140) than the amount burned by the operated animals (190). It is concluded that a high fat diet depresses the oxidation of glucose in normal, but not in hypophysectomized rats.—M. H. F.

Pregnancy diagnosis by chemical test of urine (Die chemische Schwangerschaftsdiagnose aus dem Harn). Frankl, O., and P. Engel, Zentralbl. f. Gynäk. **60:** 2645. 1936.

The Visscher and Bowman test has been tried on 200 cases, and found to be unreliable for either pregnant or non-pregnant cases.—E. L. S.

Pituitary basophilism (Cushing's syndrome). Report of a verified case, with a discussion of the differential diagnosis and treatment. Freyberg, R. H., P. S. Barker, L. H. Newburgh and F. A. Coller, Arch. Int. Med. **58:** 187. 1936.

This is the report of a verified case of pituitary basophilism showing the classic clinical syndrome, and a summary of the essential features of other verified cases reported since Cushing's review in 1933. The authors point out the resemblance of the clinical manifestations of

other lesions, especially cortical adrenal neoplasm or hyperplasia, to "Cushing's syndrome" of pituitary basophilism. The results of roentgen therapy directed to the hypophysis in cases of Cushing's disease are reviewed. This study indicates that roentgen therapy should be intensively and persistently pursued in cases of pituitary basophilism.—I. B.

The obesity and energy exchange in a varified case of pituitary basophilism. Freyberg, R. H., and L. H. Newburgh, Arch. Int. Med. **58:** 229. 1936.

In an obese patient with verified pituitary basophilism undernutrition caused loss of weight exactly as predicted for a normal person, thus demonstrating a normal energy exchange and the absence of any unusual metabolic feature as the cause of the obesity. The unusual configuration in this patient was chiefly the result of an underlying skeletal deformity and was not primarily due to adiposity.—I. B.

A new, rapid, economical test for pregnancy and certain gynecological conditions. Gilfillen, C. G., and W. K. Gregg, Am. J. Obst. & Gynec. **32:** 498. 1936.

Patients who are pregnant, or who retain viable placental tissue do not react locally to intradermal Antuitrin-S; others do. The reactions appear in a half hour usually. A résumé of a number of cases shows a high degree of accuracy, with definite errors resembling results with the usual Friedman and Aschheim-Zondek tests.—E. L. S.

Effects of two hypophyseal gonadotropic hormones on the reproductive system of the male rat. Greep, R. O., H. L. Fevold and F. L. Hisaw, Anat. Rec. **65:** 261. 1936.

The gonods of hypophysectomized and normal immature male rats underwent enlargement as a result of either FSH or LH treatment. The structural response of the testicles of these 2 hormones, however, was quite different. The FSH stimulated rapid proliferation of the seminiferous epithelium but failed to alter the interstitial cells. Contrawise, LH effected little if any proliferative activity in the tubules but did produce excessive hypertrophy of the interstitial tissue. The male secondary sexual structures remained unchanged when FSH was given but responded strongly to LH treatment due to the male sex hormone presumably secreted by the hypertrophied interstitial cells. Enlargement of the external genitalia, priapism and spontaneous ejaculations were also features of LH action. Rats treated with FSH and LH simultaneously showed both tubular proliferation and interstitial cell hypertrophy but the accessory structures attained a larger size than would be produced by an equivalent amount of LH acting alone.—Authors' Abst., Wistar Bib. Service.

Rapid method of preparing solutions of gonadotropic substance of pregnant mare's blood. Gustus, E. L., R. K. Meyer and O. R. Woods, Proc. Soc. Exper. Biol. & Med. **34:** 54. 1936.

The use of the Katzman-Doisy benzoic acid procedure on the serum of the pregnant mare resulted in very satisfactory, though not quantitative, yields of the active material.—M. H. F.

Function of pituitary grafts in mice. Hill, R. T., and W. U. Gardner, Proc. Soc. Exper. Biol. & Med. **34:** 78. 1936.

Two instances are described in which it was possible to maintain functional adrenals and gonads for several months after intratesticular transplants of mouse pituitaries into hypophysectomized recipients.—M. H. F.

The pigmentary effector system, VII: the chromatic function in elasmobranch fishes. Hogben, L., Proc. Roy. Soc. London, s.B., **120:** 142. 1936.

Total removal of the pituitary gland, or of the neuro-intermediate lobe alone, in various species of Scyllium and Raia, resulted in complete pallor persisting untill death (survival 2 months or more). Posterior-lobe extracts in which pressor and oxytocic activity had been destroyed by exposure to alkali were still capable of causing melanophore expansion. The ability of the animals to adapt themselves to a white background was lost after removal of the anterior lobe; removal of the pars ventralis alone did not abolish either the white or the black background response.—D. L. T.

The action of the posterior pituitary hormone on intra-ocular pressure (Die Wirkung der Hormone des Hypophysen-Hinterlappens auf den intraokularen Druck). Holtz, P., and G. Jancke, Arch. f. exper. Path. u. Pharmak. **181:** 494. 1936.

The work of these authors was undertaken in order to study the possibilities of using pitressin to reduce intraocular pressure in glaucoma. The experiments were carried out on

normal and urethanized rabbits and cats. Intraocular pressure was measured with the Schiotz Tonometer in cases of pressure of 7.5 mg. Pupil width was determined with the Laudmann Tangentialpupilometer. Pitressin and pitocin were used in 5, 10 and 15 U. doses injected subconjunctivally, or dropped into the conjunctival sac. Pitressin produced a distinct lowering of the intraocular pressure in the normal rabbit with an accompanying prolonged narrowing of the pupil. No evidence of absorption of the pitressin into the general circulation was observed. Pitocin narrowed the pupil of the normal rabbit eye but had no effect on the intraocular pressure. The pressure lowering effect of the pitressin on the normal eye did not depend on the narrowing of the pupil, but solely upon the contraction of the blood vessels. A few experiments on the human glaucomatous eye gave a good therapeutic result.—R. Cleveland.

The hypophysis and metabolism. Houssay, B. A., New England J. Med. **214:** 961. 1936.

The anterior pituitary has a tonic action on the basal metabolism, more particularly through maintenance of the thyroids. The neuro-intermediate lobe regulates renal water secretion. The anterior lobe influences blood-iodine concentration and maintains the plasma potassium at a normal level. It stimulates endogenous protein catabolism and influences nucleoprotein metabolism. It has a definite stimulatory action on ketonuria although its action on the disposition and consumption of fats varies among species. The pituitary is one of the most important metabolic regulatory organs, and, in some species, is of vital necessity. Growth, endocrine functions, reproduction, and general metabolism are under anterior pituitary dominance.—W. Freeman.

Carbohydrate metabolism. Houssay, B. A., New England J. Med. **214:** 971. 1936.

In the absence of the anterior pituitary gland there is a marked decrease in glycosuria during fasting in diabetes and the new formation of sugar from protein is diminished. Hypophysectomized animals become hypoglycemic and frequently die while sugar therapy produces spectacular improvements. Diabetes in hypophysectomized animals is less intense and they are able to utilize sugar. Excess of the secretion of the anterior lobe aggravates diabetes and in normal animals causes a hyperglycemia and diabetes. It increases the resistance to insulin. This action is direct, occurring even in the absence of the pancreas, gonads, thyroid or adrenals. —W. Freeman.

Asthenia hypophysopriva. Houssay, B. A., New England J. Med. **214:** 1023. 1936.

In the advanced stages of pituitary insufficiency there is a neuromuscular asthenic syndrome which occurs constantly, severely and characteristically in the toad, during certain stages in the rat, and in a less marked form in the dog. It is well developed in human cases of pituitary cachexia. The syndrome arises mainly from functional changes in the central nervous system, the peripheral motor changes playing only a secondary role. The functional changes coincide with general nutrition changes (decrease in blood sugar, glycogen and endogenous nitrogenous catabolism, and an increased sensitivity to insulin). The adrenal insufficiency which occurs in cases of pituitary insufficiency probably increases the asthenia but it is not exclusively of this origin.—W. Freeman.

Hypophysis and blood pressure. Houssay, B. A., New England J. Med. **214:** 1086. 1936.

The posterior lobe of the pituitary contains vaso-pressor substances which play an important role in the maintenance of the blood pressure as well as arterial and capillary tone. In pituitary cachexia in man, however, the hypotension appears to be due principally to a lack of anterior lobe activity. The existence of hypertension due to acromegaly is questionable but hypertension is prominent in pituitary basophilism which might well be due to adrenal hypersecretion. The theory that the cause of hypertension in cases of eclampsia and essential hypertension is overactivity of the posterior lobe is without adequate foundation. The diencephalon is not essential for the maintenance of blood pressure.—W. Freeman.

Certain relations between the parathyroids, the hypophysis and the pancreas. Houssay, B. A., New England J. Med. **214:** 1128. 1936.

In the presence of pituitary insufficiency in the dog, there is a cellular atrophy in the parathyroid glands with zones of accumulated nuclei, which simulate cords, and occasionally acellular areas. This may be due to a general nutritive lack or a lack of the parathyrotropic hormone. Anterior pituitary extract increases the size and number of cells of the parathyroids and also raises the blood calcium. This latter does not occur if the parathyroids had been

previously removed. Pituitary extracts do not prevent or cure the hypocalcemia and tetany due to parathyroidectomy. In pancreatic insufficiency there results a protoplasmic disintegration of the cells of the parathyroids; the blood calcium level is slightly lowered and the blood phosphorus increases. Insulin prevents the blood-calcium fall but does not repair the damage if it has already been established. In hypophysectomized-pancreatectomized dogs, lesions of the parathyroids are found side by side which are peculiar to both operations separately. The proper functioning of both the pituitary and pancreas is necessary for maintaining the integrity of the parathyroids. The parathyroid changes due to hypophysectomy are due to a parathyrotropic insufficiency whereas the changes brought about by pancreatectomy are probably due to general nutrition changes brought on by the resulting diabetes.—W. Freeman.

The hypophysis and resistance to intoxications, infections and tumors. Houssay, B. A., New England J. Med. **214:** 1137. 1936.

The existence of a direct antitoxic or anti-infectious action of the pituitary gland or of any of its secretions has not been established but the gland can modify the resistance of the animal by its metabolic action and through the thyroid or adrenal glands. Hypophysectomy causes the animal to tolerate anesthetics and hypnotics poorly and become more susceptible to infections, poisons, blood-pressure-lowering drugs and blood-sugar-lowering drugs. It also provokes a decrease in phagocytosis and an accelerated antitoxin formation. Agglutinin production is not disturbed. It retards but does not prevent tumor growths but does diminish the number of metastases. The urine of cancerous patients has a powerful gonadotropic activity. Pituitary extracts can accelerate the growth of certain neoplasms.—W. Freeman.

Influence of the pituitary and the adrenals on pancreatic diabetes in toads (Influencia de la hipófisis y la suprarrenal sobre la diabetes pancreática del sapo). Houssay, B. A., and A. Biasotti, Rev. Soc. argent. de biol. **12:** 102. 1936.

Total destruction of both adrenals produced a decrease in blood sugar (31-30% below normal in 24 hrs.) and a slight decrease in liver and muscle glycogen; the implantation of the glandular lobe of the pituitary raised the blood sugar in these animals, but not to normal. Hypophysectomy (glandular lobe, without anaesthesia) produced only a slight decrease in the blood sugar, 24 hrs. after operation, and no variation in the liver and muscle glycogen. Pancreatectomy increased the blood sugar to a diabetic level in 24 hrs. Extirpation of the anterior pituitary diminished considerably the severity of pancreatic diabetes; implantation of 1-2 glandular lobes raised the glycemia to a diabetic level; implantation of toad adrenal and injection of cortin did not increase the blood sugar in these animals. Destruction of the adrenals attenuated considerably the hyperglycemia due to pancreatectomy; injection of cortin did not counteract this attenuation; injection of anterior pituitary lobe raised the blood sugar to a diabetic level in these animals. Simultaneous removal of the adrenals and glandular lobe attenuated pancreatic diabetes in the same degree or only slightly more than removal of only one of the glands; injection of anterior pituitary lobe increased the blood sugar to a diabetic level in these animals; cortin and implantation of adrenals did not modify the blood sugar, nor did they increase the action of anterior pituitary lobe. The diabetogenic action of the pituitary is independent of the adrenals in this species.—J. T. L.

Glycogen and hypophysis (Glucógeno e Hipófisis). Houssay, B. A., A. Biasotti and R. C. Dambrosi, Rev. Soc. argent. de biol. **12:** 185. 1936.

Hypophysectomised puppies and adult dogs, 3 to 9 month after operation had average liver and muscle glycogen values below that of normal controls, but in some well-fed individuals high values were found. Fasting reduced liver glycogen more rapidly in hypophysectomised animals than in normal controls. Adrenalin and insulin hypoglycemia diminished liver glycogen less in the hypophysectomised than in controls. Resynthesis of muscle glycogen following tetanization after 1 hour rest was normal. Hypophyso-pancreatectomized animals had high liver glycogen except in cases which had marked hyperglycemia (above 0.3 gm.%); injection of glucose caused a considerable increase in the liver glycogen. Anterior pituitary diabetogenic extracts produced an increase in liver glycogen in dogs when the blood sugar rose above 0.18 gm.%; extirpation of the abdominal sympathetic, section of the splanchnic nerves, thyroidectomy, hypophysectomy and castration did not alter this effect. Cases which had no hyperglycemia, had slightly less liver glycogen than the controls. Glucose injection into dogs made diabetic by treatment with anterior pituitary extract produced less increase in liver glycogen than in the controls; the difference was less marked in thyroidectomized and hypophysectomized

animals. Thyrotropic anterior pituitary extract decreased liver glycogen in guinea pigs. A critical examination of all experimental data on the subject is made.—J. T. L.

Anterior-pituitary diabetes and pancreatic endocrine function (Diabetes ántero-hipofisaria y función endócrina pancreática). Houssay, B. A., and V. G. Foglia, Rev. Soc. argent. de biol. **12:** 237. 1936.

In a new and numerous series of dogs the diabetogenic activity of anterior pituitary extract was confirmed. Hepatectomy produced an immediate fall in the blood sugar of anterior-pituitary diabetic animals, to subnormal levels. A pancreatic vascular graft which produced a fall in the pancreatic diabetic hyperglycemia, did not produce a fall in blood sugar in anterior-pituitary diabetes. The pancreas from dogs with anterior-pituitary diabetes secretes less insulin than the normal, since when grafted in the neck of pancreatectomized dogs the blood sugar either did not fall or did so less rapidly and did not reach the normal level. The dogs are insulin resistant since 2 U. intravenously produced a less marked and prolonged fall in blood sugar than in normal or pancreatic-diabetic dogs. This is due to a change in the state of the tissues, since bleeding and transfusion of an equivalent amount of normal blood altered little or not at all the response to insulin; transfusion of blood from a dog with anterior-pituitary diabetes to a normal dog did not significantly alter the response to insulin. Anterior-pituitary diabetic animals are in an absolute (less secretion) and relative (insulin resistance) state of hypoinsulinism.—J. T. L.

Experimental diabetes insipidus in the monkey. Ingram, W. R., C. Fisher and S. W. Ranson, Arch. Int. Med. **57:** 1067. 1936.

The authors operated on a group of monkeys and the subsequent occurrence of diabetes insipidus in 2 of them is reported. One showed a decided increase in the average output of urine, the average volume before operation being 266 cc., which corresponds closely with the average for 7 normal monkeys, and that after operation being 749 cc. The other showed more moderate polyuria, the output of urine after operation averaging 485 cc. The remaining monkeys showed no significant increase; on the other hand, 4 had a diminished volume of urine after operation, while their intake of water was markedly reduced. Examination of the sectioned brain stems of the monkeys presenting polyuria showed that the lesions were placed so as to interrupt the supra-optico-hypophyseal tract, with concomitant atrophic changes in the supra-optic nuclei and in the pars posterior of the hypophysis. In the animals in which the results were negative, the lesions either did not affect this system or encroached on it so slightly that very little atrophy occurred in the supra-optic nuclei and the posterior lobe of the pituitary. In these animals lesions in the hypothalamus which differed little from those in the animals presenting diabetes insipidus but which did not interrupt the supraoptico-hypophyseal tract did not produce polyuria. In a large group of monkeys used for other experiments, lesions have been placed throughout the extent of the lateral hypothalamic area and in the medial and lateral portions of the posterior hypothalamic region, including the region of the mamillary bodies, and no tendency to polyuria has been observed.—I. B.

Pathogenesis of the Laurence-Biedl syndrome. Jenkins, R. L., and H. G. Poncher, Am. J. Dis. Child. **50:** 178. 1935.

A case is reported in an 11-year-old boy who presented obesity, undescended testes, hypogenitalism, mental deficiency and polydactylism. The various hypotheses concerning the pathogenesis are reviewed. It is impossible to account for the association of the characteristics of the Laurence-Biedl syndrome, according to the authors, on the basis of any of the hypotheses previously advanced. They present a hypothesis that the syndrome is due to the linkage of two or more genes bearing unit characters. These genes cannot carry simple dominants; they may bear simple recessives.—M. B. G.

The action and fate of injected posterior pituitary extracts in the decapitated cat. Jones, A. M., and W. Schlapp, J. Physiol. **87:** 144. 1936.

After intravenous injection into decapitated cats (number not given) of posterior pituitary extract (10 mg. of dried gland per kg. body weight) the pressor and oxytocic activities of the circulating plasma were analysed by biological assay. It was found that both were absent after 2 hours and that 85% was lost in the first 20 minutes. About 30% of the injected pressor principle appeared in the urine during the time the active principle was disappearing from the blood stream. During this period small test pressor doses gave a sub-normal response until the residual

large dose had been eliminated from the blood stream. In vitro experiments, in which the oxytocic and pressor activities of posterior pituitary were destroyed by glycerol tissue extracts, suggested that their disappearance from the circulation involves destruction as well as excretion.—R. L. Z.

Studies on water metabolism in normal and hypophysectomized frogs. Jones, M. E., and F. R. Steggerda, Am. J. Physiol. **112:** 397. 1935.

A comparative study of the water uptake by 10 normal and 11 hypophysectomized frogs (1-4 weeks previous to time of experiment) was made following injection of pituitrin (Parke-Davis). Following a control weighing they were injected with pituitrin (0.1cc. per 10 gm. body weight) and weighed thereafter at hourly intervals for 6 hours. In all cases the frogs were kept nearly submerged in water. The average change in weight resulting from the uptake of water showed that the normal frogs increased approximately 18% in weight, reaching their highest point in 3 to 4 hours, whereas the hypophysectomized frogs when similarly treated gave practically no response to pituitrin injection. Cerebral injury does not alter the ability of pituitrin to produce weight changes.—F. R. Steggerda

Relation of anterior pituitary to the volume of islet tissue in the male rat. Krichesky B., Proc. Soc. Exper. Biol. & Med. **34:** 126. 1936.

In male rats, hypophysectomized 30-60 days previously, the volume of islet tissue (determined by planimeter tracings of drawings of sections) per gram of body weight was markedly increased over that of the controls. The injection of crude alkaline extract of A.P. 4 times weekly into hypophysectomized males resulted in an islet/body weight ratio between that of the controls and of the operated, uninjected animals.—M. H. F.

Action of the extracts of the posterior hypophysis on the gastric secretions. The therapeutic value of the extracts (Action des extraits de post-hypophyse sur la sécrétion gastrique valeur thérapeutique de ces extraits). Langeron, L., M. Paget and A. Danès, Rev. franç. d'endocrinol. **14:** 28. 1936.

Pituilobine (Byla) in doses of 40 I.U. was used for a subcutaneous injection. In 10 patients, the immediate action and in 16 patients the delayed action of the Pituilobine were studied. There was a temporary inhibition on the gastric secretion, both the free HCl and total acidity. In a series of 12 daily injections variable effects were noted. It was considered premature to use the extract of the posterior hypophysis as a specific medication for hyperchlorhydria.—J. P. P.

The effect of tissue enzymes on posterior pituitary extract. Larson, E., J. Pharmacol. & Exper. Therap. **57:** 132. 1936.

Kidney, liver, muscle or blood tissue preparations of dogs, cats or rabbits inactivated the pressor and oxytocic principles of pituitary extract due to the ereptases present.—E. L.

New developments on the fate of prolan injected into the rabbit (Nouvelles recherches sur le sort du prolan injecté à la lapine). Lipschütz, A., A. Fuente-Alba and T. Vivaldi, Compt. rend. Soc. de biol. **118:** 226. 1935.

By the method described in previous papers the quantity of prolan present in the blood several hours after intravenous injection has been determined with the object of constructing the curve of its disappearance from the blood. The prolan content of the blood falls rapidly after injection; in 2 hours about 30%, and in 4 hours about 50% of the injected prolan have disappeared from the blood. At 10 and even 18 hours after injection prolan is still to be found in the blood but about 90% or more has disappeared already at 12 hours. The curve of disappearance of the prolan from the blood is logarithmical.—A. Lipschütz

On the fate of prolan after intravenous injection into the rabbit (Sur le sort du prolan injecté à la lapine par voie intraveineuse). Lipschütz, A., and T. Vivaldi, Compt. rend. Soc. de biol. **116:** 87. 1934.

One hundred R.U. of prolan extracted from human pregnancy urine was injected intravenously into adult female rabbits. The quantity of prolan present in 1 cc. of blood of these animals immediately after injection was calculated from the data of Dreyer and Ray for the volume of blood as a function of body weight. Several hours later samples of blood of the injected animals were transfused into 26 adult rabbits to determine the minimal quantity of

transfused blood which was still able to produce hemorrhagic follicles and to calculate the percentage of prolan still present in the blood at that hour. About 70 to 80% of the prolan injected had disappeared from the blood in the course of 6 to 8 hours after injection.—A. Lipschütz.

Relation of the Cushing syndrome to the pars intermedia of the hypophysis. MacCallum, W. G., T. B. Futcher, G. L. Duff and R. Ellsworth, Bull. Johns Hopkins Hosp. **56:** 350. 1935.

A 25-year-old Polish Jewess complained of symptoms which began roughly about a year prior to her death, before which she had been in reasonably good health although she had always been about 40 pounds overweight. Her menstrual flow had gradually ceased. Pads of fat, together with reddish-purple striae of the skin, appeared in her face, shoulders, chest and abdomen. Her legs, up to her knees, would become swollen towards the end of each day. Later she developed polyphagia, polyuria and polydypsia of 2 gallons a day. She had frequent attacks of dizziness and night sweats. Her hair began to fall out in large quantities although she did not have hirsutism; her hair was of normal feminine distribution. Her blood pressure was 210/150. Skiagrams showed demineralization of the skull. Her blood sugar was 225 mg.; blood calcium, 11.2 mg.; blood phosphorus, 4 mg.; and she excreted about 81 gm. of sugar in her urine daily. Prolan B could not be detected in her urine. She developed a fatal renal infection. Post-mortem examination revealed multiple abscesses of the myocardium, lungs, kidneys, and right adrenal medulla, and an acute splenitis. The skull was so soft it was easily cut with a knife although the parathyroid bodies appeared normal both grossly and microscopically. The left adrenal gland weighed 14.75 gm. and histologically showed small round cells infiltrating the medulla. No other organic abnormalities could be found except a small spherical mass in the anterior pituitary, measuring 0.7 cm. in diameter and projecting back to the pars intermedia. Microscopically, the tumor was continuous with the pars intermedia and took the basophilic stain, using the routine staining methods. With Cowdry's copper-hematoxylin stain, however, the tumor did not take the stain (unlike the basophilic cells of the anterior lobe but like the cells of the pars intermedia).—W. Freeman.

Changes in the hypophysis in beriberi (Über Veränderungen der Hypophyse bei Beriberi). Marburg, O., Wien. Arch. f. inn. Med. **29:** 1. 1936.

Six cases are described with autopsy findings. In general the hypophysis is found to be reduced in size with degenerations and with a reduction or disappearance of basophil elements. It cannot be stated whether the hypophyseal changes are primary, or are secondary to changes in other glands or whether the various glandular and internal organs are affected coördinately by the disease processes involved in beriberi. The latter seems the more probable. The author holds, however, that the changes in such endocrine glands as the hypophysis and the adrenal have a definite effect on the symptoms of beriberi, increasing their severity and lending an endocrine character to them.—W. J. A.

Pituitary cachexia or Simmonds' disease (La cachexie hypophysaire ou syndrome de Simmonds). May, E., and P. Robert, Ann. de méd. **38:** 317. 1935.

The syndrome is described of a single case, a girl of 22 years. Only true pituitary extracts of high potency given in sufficient dosage offer adequate treatment. Pregnancy urine preparations are of limited value. Other glandular extracts may be of some help but do not have a definite action.—A. E. M.

Physiological activity of the posterior pituitary of blue and sperm whales. McClosky, W. T., L. C. Miller and D. H. LeMessurier, J. Pharmacol. & Exper. Therap. **57:** 132, 1936.

The pressor and antidiuretic potencies of the blue-whale and sperm-whale pituitaries were equal to those of beef pituitaries. The oxytocic activity compared to that of beef pituitaries, was 10% and 30%, respectively.—E. L.

Effects of posterior pituitary extract on the water and mineral exchange in edema. McQuarrie, I., W. H. Thompson and M. R. Ziegler, J. Pediat. **8:** 277. 1936.

The effects of sustained pituitary antidiuresis on the water and mineral balances of the body have been studied in patients suffering from edema of several types. The diuresis which follows a period of sustained pituitary antidiuresis results in a significant net loss in extracellular body water in the normal or even the dehydrated patient if the diet given is extremely low in

sodium chloride. Under similar circumstances certain edematous subjects may lose a high percentage of their edema fluid, making this a valuable therapeutic aid in such cases. Both edematous and non-edematous subjects show distinctly negative sodium and chloride balances during both the antidiuretic and diuretic periods. The potassium, phosphorus and nitrogen balances are little influenced by pitressin administration. The serum albumin tends to rise coincidently with the disappearance of the edema. Extreme depletion of serum albumin greatly impairs the effectiveness of the procedure. The use of blood transfusion preliminary to the administration of pituitary extract is therefore recommended.—M. B. G.

Bioassay of galactin, the lactogenic hormone. McShan, W. H., and C. W. Turner, Proc. Soc. Exper. Biol. & Med. **34:** 50. 1936.

Determination of the quantity of material to produce a minimal response of 50% of young common pigeons weighing 260 to 340 gm. gave more accurate and more consistent results than the previous methods of assaying by the weight of the crop gland.—M. H. F.

The influence of salt saturation upon the urinary response to pituitary (posterior lobe) extract. Melville, K. I., J. Physiol. **87:** 129. 1936.

In 22 experiments on two unanesthetized bladder-fistula dogs and 10 experiments on dogs anesthetized with sodium phenobarbital and with ureters canulated it was found that the diuretic response to posterior pituitary extract (Postlobin-V) was augmented by previous administration of sodium chloride. Potassium chloride and sodium nitrate had a similar but less marked effect, while sodium sulphate did not augment diuresis. The results are discussed in relation to a hypothesis that Postlobin-V has 'salt-mobilizing' and 'water retaining' actions, rather than 'diuretic' and 'antidiuretic' effects.—R. L. Z.

The source of the blood acetone resulting from the administration of the ketogenic principle of the anterior hypophysis. Mirsky, I. A., Am. J. Physiol. **115:** 424. 1936.

Twelve white male New Zealand rabbits, weighing approximately 2 kg. each were used. All were under nembutal anesthesia. Six were totally eviscerated and 6 used as controls. Lilly's beef anterior pituitary extract in doses equivalent to 2 gm. or more of fresh gland per kg. body weight was administered subcutaneously, hourly blood samples were drawn from the femoral artery and total acetone was determined by a modification of the Van Slyke and Fitz method. Eviscerated animals showed no blood acetone bodies at any time while controls showed considerable quantities after injection of the pituitary extract. Rabbits with all viscera except the liver removed showed blood acetone comparable to controls. It was concluded, therefore, that the ketogenic principle of the anterior hypophysis acts solely on the liver and does not stimulate the oxidation of fat in extra-hepatic tissues; the muscles do not contribute significantly to the accumulation of ketones in the blood. The production of acetone bodies is attributed to a relative decrease of carbohydrate utilization by the cells of the liver in the presence of an increased catabolism of fatty acids consequent to an accelerated hepatic glycogenolysis.—K. A. Brownell.

Responses of immature rat testes to gonadotropic agents. Moore, C. R., Am. J. Anat. **59:** 63. 1936.

Extracts of pregnancy urine (antuitrin-S), of dried sheep pituitary (pituitary hebin), of normal male urine (prospermine), as well as whole sheep pituitary suspension and pregnant mare serum have been used in low effective doses to marked overdoses on male rats at various ages from birth to maturity. Testis weight increases ranged from 0 to approximately 200% whereas seminal vesicle weight increases ranged up to approximately 5000%. Spermatogenesis, as judged by attainment of a definite metamorphosing spermatid stage just short of the completed spermatozoon, was not hastened by any treatment (if at all) by as much as 2 to 3 days. Sensitivity to gonadotropic agents is great at ages from birth to about 35 days and declines with ascending ages; mature testes are affected relatively little.—Author's Abst., Wistar Bib. Service.

Posterior pituitary lobe and imbibition in batrachians (Lóbulo posterior de hipófisis e imbibición de los batracios. II. Mecanismo de su acción). Novelli, A., Rev. Soc. argent. de biol. **12:** 163. 1936.

In experiments on Bufo arenarum the animals were submerged in distilled water and solutions of NaCl from 0.25% to 1.2% until constant weight was obtained. They were then in-

jected with 10 U. posterior pituitary extract (5 mg. standard powder) per 100 gm. and weighed 7 hours later; the increase in weight was greater in water (15-18%) and inversely proportional to the concentration of the NaCl solution, with 0.7% and more, no increase in weight occurred. When the animals had been kept in equilibrium with an 0.5-0.6% solution and were passed to one of 1.0-1.2% they lost weight (3.8%) even when injected with the extract, hence the osmotic effects are stronger than the activity of the extract. Destruction of the brain and spinal cord did not modify the action of the extract; thyroidectomy decreased it only a little. Very large doses of extract (50 mg. standard powder) produced the same increase in weight (13%) as smaller (5 mg.) doses (12%). When the skin of the hind quarters was removed injection of extract was not followed by an increase in weight as in the controls. In other experiments skin sacks were made by removal of muscle and bone, taking care to leave the skin circulation intact; those injected darkened and in the sacks greater amounts of water were found than in the controls in similar conditions. In another series the cloaca was emptied and sewn up and the animals submerged as usual; 2 and 6 hours later they were weighed and the urine measured; injected animals secreted considerably less urine (oliguric action of extract) but had a much larger increase in weight. From these experiments it is deduced that the skin permeability increases under the influence of posterior pituitary extract.—J. T. L.

The action of the preparations from the posterior lobe of the pituitary gland upon the imbibition of water by frogs. Oldham, Frances K., Am. J. Physiol. **115:** 275. 1936.

The relative effectiveness of the different constituents of posterior lobe extracts in producing imbibition of water by frogs was studied, using 2 different preparations of oxytocic and pressor hormones and a melanophore-dilating preparation relatively free from the other constituents. The last substance was found to be inactive while the potency of oxytocic substances proved to be between 2.5 and 5 times that of pressor substances. The action was found to compensate for, but not to affect the elimination of, saline.—R. R. Durant.

Comparison of prolan bioassays in teratoma and other conditions. Owen, S. E., and M. Cutler, Am. J. M. Sc. **192:** 61. 1936.

Using the Aschheim-Zondek mouse test, quantitative determinations of the urinary prolans in 125 cases of suspected teratoma testis have been made. In general, patients suffering with teratoma testis were found to excrete more than 100 mouse units of the prolans per liter of urine. False negatives or false positives occurred in less than 10% of the cases; thus the test is as accurate as the usual laboratory test. Original teratomas, local recurrence or metastases were associated with increasing prolan findings as displayed by serial assays. A marked diminution in the prolan output usually followed irradiation or surgery.—R. G. H.

Extirpation of pituitary gland on arterial blood pressure of dogs with experimental hypertension. Page, I. H., and J. E. Sweet, Proc. Soc. Exper. Biol. & Med. **34:** 260. 1936.

In dogs with experimental hypertension, produced by renal ischemia, hypophysectomy sharply reduced the blood pressure toward the normal level. Yet, hypophysectomy did not entirely prevent the rise in pressure if the renal ischemia was produced afterwards. The hypertension in such animals, however, was transient.—M. H. F.

Diuresis in normal and hypophysectomised toads (Estudios sobre el metabolismo hídrico en el Bufo arenarum, Hens. IV. La diuresis de los sapos hipofisoprivos normales dejados en seco o inyectados con solución fisiológica). Pasqualini, R. Q., Rev. Soc. argent. de biol. **12:** 37. 1936.

Hypophysectomized toads kept in dry conditions had a considerably greater diuresis than normal controls. In 3 days the loss of weight was 14% and 13.3% of the initial maximal hydrated weight; diuresis was responsible for 6.9% of the loss and evaporation for 7.1%; the corresponding figures for the controls were 3.1% and 10.2% (average of 34 operated and 46 normal animals). The difference in diuresis was most pronounced on the first day. Injection of normal saline into the abdominal vein produced more abundant diuresis in toads hypophysectomized from 3 to 22 days previously than in the controls; the difference was 82, 84 and 114% in the 1st, 2nd and 3rd hours. In 3 hours the hypophysectomised had eliminated 76.5% of the fluid injected and the controls only 40.5% (average of 16 animals in each series). After extirpation of the principal lobe of the hypophysis the animals responded as the controls in both experiments. From these results and from previous experiments it is concluded that the kidney

is the most important factor in hypophyseal diuresis, the skin and tissues playing secondary roles.—J. T. L.

Water metabolism of the rat following removal of the anterior lobe of the hypophysis. Pencharz, R. I., J. Hopper, Jr. and E. H. Rynearson, Proc. Soc. Exper. Biol. & Med. **34:** 14. 1936.

Removal of the hypophysis in female rats produced only a temporary polyuria and polydypsia. Ablation of the anterior lobe alone produced a temporary reduction in water intake, with a return to normal in about a week. Removal of the posterior lobe, with little or no injury to the anterior lobe, produced a permanent polyuria and polydypsia (still observed at 6 months.) However, implantation of anterior lobes into completely hypophysectomized rats did not produce diabetes insipidus in the treated animals.—M. H. F.

The inhibition of water diuresis by pituitary (posterior lobe) extract and its relation to the water load of the body. Pickford, Mary, J. Physiol. **87:** 291. 1936.

From data obtained from previous water absorption curves, the water load in cc. was calculated for bitches (number not given) after administration of 200 or 250 cc. of warm tap water by stomach tube. Measurement of urine flow after intravenous post-pituitary extract showed a roughly inverse proportionality between water load and percentage inhibition in the rate of urine flow.—R. L. Z.

Mammary gland development in the hypophysectomized albino rat. Reece, R. P., C. W. Turner and R. T. Hill, Proc. Soc. Exper. Biol. & Med. **34:** 204. 1936.

The injection of progynon B subcutaneously in doses from 25 to 500 U. daily for 15 to 45 days failed to induce any mammary gland growth in male or female immature, hypophysectomized rats. These results are not in accordance with results obtained with oestrin injections in hypophysectomized animals by other authors under different conditions from those obtaining in these experiments.—M. H. F.

Polyuria and polydipsia (diabetes insipidus) and glycosuria resulting from animal experiments on the hypophysis and its environs. Reichert, F. L., and W. E. Dandy, Bull. Johns Hopkins Hosp. **58:** 418. 1936.

The topographical relations in man and dog differ so radically as to make impossible transfering results of experiments on brain injuries from the one species to the other. Experiments on dogs indicate that diabetes insipidus is not due to the absence of any hypophyseal hormone, nor do the hypophysis, hypothalamus or any part of the base of the brain have any demonstrable bearing on the production of glycosuria in dogs.—A. G.

Morphogenetic effect and biological standardization of corticotropic substance (Zur morphogenetischen Wirkung und biologischen Eichung des kortikotropen Wirkstoffes). Reiss, M., J. Bálint, F. Oestreicher and V. Aronson, Endokrinologie **18:** 1. 1936.

Within 10 days after hypophysectomy there develops in the adrenal of the rat a wide 'sudanophobic' zone which can be restored to normal by corticotropic substance of the anterior hypophysis. The development of this zone has been shown to be specific for hypophysectomy and its restoration to be caused by corticotropic substance alone. A method for standardizing corticotropic hormone has been devised which uses hypophysectomized rats weighing 80-120 gm. In 10-20 days after operation one adrenal is removed and frozen sections stained with sudan are prepared and examined for the development of the 'sudanophobic' zone. The extract to be standardized is then divided into 16 doses which are injected twice daily for 8 days. The remaining adrenal is sectioned and then compared with the first. One unit is defined as the smallest amount of hormone, administered as above, which will produce definite lipoid regeneration.—W. J. A.

A study of anti-thyrotropic activity. Rowlands, I. W., and A. S. Parkes, Proc. Roy. Soc. London, s.B. **120:** 114. 1936.

The unit of thyrotropic hormone is defined as the amount required to double the weight of the thyroid (normally 30 mg.) in immature female guinea-pigs. The serum of normal sheep and horse, but not of cow, goat, or rabbit, showed some slight power to inhibit this reaction. Marked inhibitory properties developed in the serum of rabbits injected with 10 units daily:

the amount of thyrotropic extract inhibited by 2 cc. serum was 0.8 mg. in 3.5 weeks, 2.8 mg. in 5 weeks, 3.4 mg. in 7 weeks, and 4 mg. (1 unit) in 10 weeks, after which it remained constant up to 18 weeks. The effect of combining thyrotropic extract and anti-thyrotropic serum in various doses is shown in curves and tables. Histological study of thyroid activity supports the results based on thyroid weight. These experiments confirm those of Collip and co-workers.—D. L. T.

Carbohydrate levels in fasted and fed hypophysectomized rats. Russell, J. A., Proc. Soc. Exper. Biol. & Med. **34:** 279. 1936.

Although fasting carbohydrate levels of operated animals is within normal limits, the values for liver glycogen, muscle glycogen and blood glucose are far below normal after a fast of 8 or 18 hours. Moreover, the feeding of 1 gram of starch to such animals did not raise the carbohydrate levels to the same height as in the normal animals fed similarly. An additional gram of starch however, 4 hours after the first, raised muscle glycogen and blood glucose to normal values, but liver glycogen lagged behind.—M. H. F.

Quantitative Friedman test in hydatidiform mole and vomiting of pregnancy. Schoeneck, F. J., Am. J. Obst. & Gynec. **32:** 104. 1936.

Using standard rabbits, and varying the dose of urine from 0.025 cc. up, a series of titrations was carried out on 67 urine specimens from 41 pregnant women. Greatest potency was found between 6th and 10th weeks of gestation. The strongest reaction was represented by a positive from 0.05 cc. and the weakest from 5.0 cc. Study of the vomiting cases suggests that this disturbance is in proportion to the intensity of the gonadotropic strength of the urine voided. Four cases of hydatidiform mole and one of chorionepithelioma were studied. Two mole cases had positive reactions with 0.006 cc., thus being more productive than any of the pregnancies, but the other 2 mole were no more productive than uncomplicated pregnancies. These latter 2 on section proved to have very inactive tissue, suggesting that they were degenerating. The chorionepithelioma was one of the least active specimens studied.—E. L. S.

Pituitary growth factor. Shelton, E. K., California & West. Med. **45:** 20. 1936.

After some years' experience with 2 preparations of bovine adenohypophysis relatively high in growth-promoting principles, an attempt was made to evaluate their clinical usefulness. Enough has been learned to warrant the conclusion that a pure, potent hypophyseal growth hormone would be a boon to medicine. The preparations employed are sufficiently active to stimulate growth during the growth period, but not at a rate sufficiently above normal to rehabilitate the classic dwarf. After the growth period they have little effect even when the epiphyses remain open. It is still clinically impracticable to prevent dwarfism when the growth increment is 50% or more deficient. While the growth-stimulating property of these preparations does not warrant enthusiasm, the preparations are clinically useful.—I. B.

Effect of cattle anterior pituitary extract on bone and cartilage of the joint (Acromegalic arthropathia). Silberberg, M., Proc. Soc. Exper. Biol. & Med. **34:** 333. 1936.

After 10 to 14 injections of acid anterior pituitary extract into guinea pigs, there was seen proliferation of joint cartilage, followed by liquefaction and microscopic ulceration of the joint surface.—M. H. F.

Effects of extract of cattle anterior pituitary on endochondral ossification in thyroidectomized young guinea pigs. Silberberg, M., Proc. Soc. Exper. Biol. & Med. **33:** 554. 1936.

The injection of cattle pituitary (acid) extracts into young, thyroidectomized guinea pigs produced an acceleration of bone and cartilage growth, and early epiphyseal closure. This effect was even more pronounced than had been obtained with normal, injected animals. It is therefore concluded that the presence of the thyroid gland is not essential for the action of such extract in promoting growth of bone and cartilage.—M. H. F.

Effect of acid extract of cattle anterior pituitary on bone repair in thyroidectomized guinea pigs. Silberberg, M., and R. Silberberg, Proc. Soc. Exper. Biol. & Med. **34:** 108. 1936.

The daily injection of acid extract of cattle A.P. into thyroidectomized guinea pigs following experimental fractures, resulted in a marked acceleration of the processes concerned

in bone repair over that seen in the thyroidectomized controls. The bone repair in the latter was slower than normal.—M. H. F.

The evaluation of the hypophyseal factor in the interpretation of endometrial changes. Simpson, J. W., E. T. Ellison, D. Phelps and J. C. Burch, Am. J. Obst. & Gynec. **32:** 129. 1936.

To determine whether the anterior pituitary has any direct effect on endometrium or only via the follicles and corpus luteum, the authors used spayed guinea pigs. Using Evans' alkaline extract there was slight positive effect and this was still found when the extract was heat treated so that all gonadotropic potency was lost. This suggests that the estrin known to occur in such anterior lobe extracts is responsible for the slight endometrial changes. Also they were able to produce the glandular cystic hyperplasia in the endometrium by use of pregnancy urine in spayed animals with partial or complete hypophysectomy, demonstrating that the anterior lobe is not necessary for the endometrial response although the response is greater with intact pituitary. They conclude that the anterior lobe need not be considered a direct factor in clinical evaluation of the endometrium.—E. L. S.

Influence of posterior pituitary extracts on sodium balance in normal subject and in patient with diabetes insipidus. Smith, F. M., and E. M. MacKay, Proc. Soc. Exper. Biol. & Med. **34:** 116. 1936.

In a normal person on a constant diet the injection of 0.5 cc. of surgical pituitrin twice daily produced a negative sodium and chloride balance, and a positive potassium balance as compared with control periods (based on urinary excretion of these elements, assuming other routes relatively constant). This injection, however, did not influence the urinary volume of the normal subject. In the patient with diabetes insipidus a similar injection markedly reduced the water output, but produced a positive balance of sodium, potassium and chloride.—M. H. F.

Effect of hypophysectomy on blood sugar of Rhesus monkeys. Smith, P. E., L. Dotti, H. H. Tyndale and E. T. Engle, Proc. Soc. Exper. Biol. & Med. **34:** 247. 1936.

Following a 16 hour fast the blood sugar level in hypophysectomized monkeys averaged 59 mg. % (33 observations; extremes 20-90), while the value in normal monkeys was 110 mg. % (53 observations; extremes of 90-150). Hypoglycemic collapse happened frequently in the operated animals after an 18 hour fast.—M. H. F.

Response of normal and hypophysectomized Rhesus monkeys to insulin. Smith, P. E., H. H. Tyndale, L. Dotti and E. T. Engle, Proc. Soc. Exper. Biol. & Med. **34:** 250. 1936.

The injection of 1/16 U. of insulin per kilo reduced the fasting blood sugar of hypophysectomized monkeys by 35% of the preinjection level, as against a 14% reduction in normal, fasted monkeys. In 3 monkeys in which less than 1/4 of the gland had been left post-operatively, the blood sugar response to the insulin injection was like that of the normal animal, rather than the completely hypophysectomized animal; i.e. a reduction of 15%.—M. H. F.

A centre, or centres, in the hypothalamus controlling menstruation, ovulation, pregnancy, and parturition. Theobald, G. W., Brit. Med. J. **1:** 1038. 1936.

On the basis of indirect circumstantial clinical evidence the author postulates that there are one or more centres in the brain, probably in the hypothalamus, which control menstruation, ovulation, pregnancy and parturition. He suggests that this centre receives afferent stimuli from (a) the ovaries (hormonal in nature); (b) the external generative organ (by nervous paths); and (c) the special senses and psyche (that is, psychological). The efferent impulses are supposed to operate through the anterior pituitary gland and through nervous impulses to the generative organs.—H. B. Friedgood.

Relationship between chloride and water excretion after pituitrin (Über den Zusammenhang zwischen Chlorid-und Wasserausscheidung nach Pituitrin). Unna, K., and L. Walterskirchen, Arch. f. exper. Path. u. Pharmakol. **181:** 681. 1936.

The relationship between chloride and water excretion in fasted and fed animals was studied in dogs after the administration of pituitrin. The urinary fistula method was used. A control experiment in which the administration of 200 cc. water increased the urinary output per unit of time and decreased the chloride content in mg.% was made. Following this experiment it was found that simultaneous administration of 200 cc. water and 0.25 Voegtlin U.

pituitrin produced a marked rise in the chloride excretion with no change in the volume of urine. In fasted dogs doses of 0.1 and 0.01 U. of pituitrin were effective in producing some increase of chloride excretion but no change in urine volume. Doses of 1 and of 3 U. pituitrin in these animals doubled the chloride output and produced an increase in the amount of urine. Animals on a high salt intake level responded to low doses of pituitrin with a much higher value for chloride excretion as well as with an increased volume of urine than did those animals on a low salt level. The authors state that the amount of urine secreted after pituitrin administration was dependent on the amount of chloride excreted from the tissues.—R. Cleveland.

Hypophyseothyrogenic adiposity and emaciation. Wahlberg, J., J.A.M.A. **106:** 1968. 1936.

The author reports a case that presented two syndrome: first adiposity and then emaciation. The main symptoms of the syndrome discussed are severe loss of weight and of appetite, constipation, amenorrhea, hypothermia, bradycardia, arterial hypotonia, hypometabolism and disturbances of the sugar metabolism. Less constant symptoms are epigastric discomfort and increased sedimentation rate of the red blood corpuscles (von Bergmann), falling of hair (Hawkinson), reversible phlebosclerosis, and an apparent tendency to acromicria (Wahlberg). The syndrome is qualitatively almost exactly like Simmonds' pituitary cachexia but is in every detail slighter and reversible, and there are no signs of an organic lesion of the hypophysis. The theory put forward, that the syndrome may be conditioned by a functional deficiency of the anterior pituitary, is supported and at the same time it is pointed out that it seems as if the thyrotropic part of the adenohypophysis function plays an important part in the pathogenesis. In support of this theory it is stated that the syndrome resembles hypothyroidism in detail, except for the absence of myxedema, and that, at least in certain cases, medication with thyroid preparations has an extraordinary effect: 3 of the author's 4 patients recovered rapidly and completely during thyroid medication. The case discussed first showed a thyrotoxicosis accompanied with adiposity, and then changed directly to the type of emaciation discussed with prompt recovery during thyroid medication. These two clinical pictures are presented as complete contrasts: probably hypophyseogenic thyrotoxicosis with adiposity and hypophyseogenic hypothyroidism with loss of weight. Very likely the first is due to hyperfunction, the latter to insufficiency of the anterior pituitary and chiefly of the thyrotropic part of its endocrine function. —Abst., A.M.A. Service.

The anatomy of the hypophysis of whales. Wislocki, G. B., and E. M. K. Geiling, Anat. Rec. **66:** 17. 1936.

The pituitary glands of the sperm whale (Physeter megalocephalus), the finback whale (Balaenoptera physalus), the blue whale (Balaenoptera sibbaldii), and the bottle-nosed porpoise (Tursiops truncatus) have been studied. In these species the adult gland is made up of a large, flattened anterior lobe and a smaller neural lobe; the latter is surrounded by leptomeninges, as well as by dura and subdural space, and is consequently entirely separated from the anterior lobe. The anterior lobe is imbedded directly in the thick dural lining the floor of the whale's cranial cavity. There is no pars intermedia or a residual lumen present. From the anterior lobe there is an extension upward of the pars tuberalis which pierces the leptomeninges to terminate by enfolding the infundibular stalk in the manner of a small clasp. The pars tuberalis is extremely vascular and is composed of small, faintly staining cells which are different from the cells of the anterior lobe. With the techniques employed, the neural process is composed of a fibrillar substance containing relatively few well-defined cells and abundant blood vessels. No nerve cells are visible in any part of the neural hypophysis. Hyaline bodies of Herring are abundantly present in the neural lobe. Because of the anatomical separation of epithelial and neural lobes in the whale, these bodies cannot be regarded as a product of any portion of the epithelial hypophysis. —Authors' Abst., Wistar Bib. Service.

The permeability of the hypophysis and hypothalamus to vital dyes, with a study of the hypophyseal vascular supply. Wislocki, G. B., and L. S. King, Am. J. Anat. **58:** 421. 1936.

The hypophysis, in contrast to the bulk of the brain, stains with acid dyes, whether easily diffusible or colloidal. The staining involves the anterior lobe, the neurohypophysis and the eminentia saccularis. The latter, which in the past has been considered a part of the tuber cinereum, deserves, on the basis of vital staining and vascular supply, to be assigned to the infundibulum of the hypophysis. In the neurohypophysis colloidal dyes are aggregated as free interstitial granules and intracellularly in glial cells. The blood supply of the pituitary of the rhesus monkey has been studied. Portal veins which arise in the hypophyseal stalk unite with

arterial branches to supply the sinusoids of the anterior lobe. The anterior lobe is drained by veins leaving the lateral borders of the lobe to enter the adjacent cavernous sinuses. Thus the blood supply is similar to that of the liver. Portal veins draining the anterior or posterior lobes via the stalk, and ultimately reaching the tuber cinereum or hypothalamic vegetative centers, have not been found. The blood supply of these latter regions is essentially independent of that of the hypophysis and its stalk. The posterior lobe of the pituitary has ample arterial and venous drainage making it to a great degree independent of the other parts of the gland.—Authors' Abst., Wistar Bib. Service.

Reaction of anterior pituitaries of immature female rats to injections of various amounts of oestrin. Wolfe, J. M., and C. S. Chadwick, Proc. Soc. Exper. Biol. & Med. **34:** 56. 1936.

Varying amounts of oestrin (10 to 200 R.U.) were injected daily into immature rats for 5 or 10 days. In all doses the injections increased the mean pituitary weight. Small doses of oestrin resulted in degranulation of basophiles with a reduction in the relative percentage of granulated basophiles. The eosinophiles were not markedly affected until much larger doses were injected. These caused degranulation of eosinophiles as well as basophiles, but resulted in no decrease in the relative percentage of granulated eosinophiles, perhaps because of the increased mitotic activity of the latter (no increased mitotic activity noted in the basophiles).—M. H. F.

The action of a synthetic oestrogenic agent on the anterior pituitary of the castrated female rat. Wolfe, J. M., Am. J. Physiol. **115:** 665. 1936.

A synthetic estrogenic compound, 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, known to possess the capacity of inducing estrual changes in the vaginae and uteri of castrated rats, was injected daily into 20 mature females over a 30-day period following castration. Such animals remained in a continuous or nearly continuous state of estrus. Pituitary weights were increased as compared with 30-day castrate controls. The structural changes characteristically developing in the pituitary following castration were prevented, accompanied by extreme degranulation of basophiles and moderate degranulation of eosinophiles. In relative percentages the eosinophiles and basophiles were reduced, the chromophobes increased. In 2 instances cystic glandular hyperplasia of the uterus was produced.—H. O. Haterius.

PANCREAS

Diagnosis and treatment of hyperinsulinism. Aitkin, L. F., M. Clin. North America **20:** 393. 1936.

This is a good review of the subject with report of an illustrative case in a married woman of 44. She presented a pancreatic adenoma of the beta type. This was successfully removed with complete cure. Outstanding features in this case were the fact that Graves' disease had followed the inception of attacks of hypoglycemia; an increased severity of hyperinsulinism followed thyroidectomy, and that a hypothyroid state followed the removal of the pancreatic adenoma. These factors suggest a compensatory action of the thyroid gland in hypoglycemic states.—I. B.

Social pathology of diabetes mellitus based on 1,142 clinical cases (Ein Beitrag zur sozialen Pathologie des Diabetes mellitus auf Grund 1,141 klinisch beobachteter Fälle). Benedict, B., and S. Kemény, Wien. Arch. f. inn. Med. **28:** 87. 1936.

By means of statistics gained from several countries diabetes is considered—particularly for the decade 1923-1933—from the viewpoints of sex, race, heredity, mortality, complications, prognosis and management. The authors are hopeful that the battle against diabetes may be productive of results comparable with the progress made against tuberculosis, syphilis and other infectious diseases. To attain these ends they believe three things necessary: 1) that physicians use all therapeutic measures available; 2) that intelligent cooperation of patients be obtained; and 3) that money be available for proper diets and treatment with insulin.—W. J. A.

Some physical and physiologic properties of the system insulin-tannic acid. Bischoff, F., Am. J. Physiol. **116:** 239. 1936.

Tannic acid precipitates insulin; in a low concentration of electrolytes the precipitate is colloidal. As measured by the incidence of insulin shock in 136 rats tested, and by blood sugar

curves in 36 rabbits, the physiologic effect of insulin is augmented by administration with tannic acid. It is suggested that this is due to delayed absorption.—F. A. Hitchcock.

Action of insulin on the respiratory quotient of phloridzinized animals and on obstructed kidneys (Azione dell'insulina sul quoziente respiratorio di animali florizinati e a reni bloccati). Bonsignore, A., and L. Cavaglione, Biochem. e terap. sper. **23:** 106. 1936.

The experimental procedure followed was to treat the animals for several days with 0.2 gm. of phloridzin per kg. of body weight. An intense glycosuria having set in the renal pelvis was ligated. Glucose was administered in varying amounts by mouth or by injection. An hour was then allowed to elapse before the gaseous metabolism and respiratory quotient was determined by Lombroso's method. The experiment proceeded for 2 hours; then insulin was administered in varying doses; then after an interval of an hour, the gaseous metabolism and respiratory quotient were redetermined. The results of these experiments showed that phloridzinized animals with renal block had a low respiratory quotient which glucose by mouth or through injection failed to elevate. Only injection of heavy doses of insulin (2/3 U. per kg.) brought the respiratory quotient to normal level.—D. T. Staffier.

Observations on the treatment of diabetes with protamine insulin in office and hospital. Bowcock, H., South. M. J. **29:** 701. 1936.

The successful use of protamine insulin by ambulant patients is illustrated by 11 cases. These varied in age between 5 and 45. They were selected because their blood sugar was difficult to control with ordinary insulin and because they could be counted on to cooperate intelligently. Five of these cases are reported in detail. In addition 17 difficult hospital cases were studied. The author summarizes: Protamine insulinate exhibits distinct advantages over regular insulin. Most patients can accomplish perfect control with a single dose daily. Almost perfect blood sugar regulation throughout the 24 hours can be secured with a single dose or a single dose plus a small simultaneous dose of regular insulin. Insulin reactions are less likely to occur and are slower in onset, giving the patient more time to take the appropriate measures for counteracting the hypoglycemia. Complicated cases of diabetes may have the benefit of more normal control. The unpleasant symptoms of hyperglycemia with its accompanying tendency to acidosis occurring in some patients may be avoided. Hepatomegaly due to fatty infiltration of the liver may be corrected. Well-trained cooperative office patients can secure the benefits of protamine insulin by observing caution and care during the period of adjustment, provided the required insulin dose is not too great. In other words with practical control and adequate cooperation, a diabetic may look forward to the benefits of essentially normal carbohydrate metabolism.—J. C. D.

The action of insulin in vitro on the blood sugar in presence of various tissues (Azione dell'insulina in vitro sul tasso glicemico in presenza di vari tessuti). Caizzone, G., Biochim. eterap. sper. **23:** 142. 1936.

The glucose content of citrated blood is not altered by insulin, and only slightly by the addition of tissue. Incubation with both insulin and tissues causes a considerable reduction of the sugar content. Liver, heart and uterus are of highest activity. The effect was especially conspicuous if blood and tissues were obtained from animals during the stage of nutritional hyperglucemia.—A. E. Meyer.

Insulin and lactic acid in the blood (Insulin et lactacidemie). Collazo, J. A., and J. Almela, Ann. de méd. **38:** 371. 1935.

The average lactic acid content in dogs' blood is 44 mg. The ingestion of 1 gm. of calcium lactate per kg. produces a mild increase of lactic acid. The increase is far larger after injection of insulin; the maximum coincides with the minimum of blood sugar. It is supposed that the adrenalin secreted under the influence of insulin causes the increase of lactic acid.—A. E. M.

The relation of pregnancy and lactation to extirpation diabetes in the dog. Cuthbert, F. P., A. C. Ivy, B. L. Isaacs and J. Gray, Am. J. Physiol. **115:** 480. 1936.

Two female dogs were depancreatized and maintained on a uniform diet and dosage of insulin which permitted a slight degree of glycosuria. Urine sugar determinations were made daily and blood sugar determinations at frequent intervals. The effect of 3 gestation and lactation periods on the amount of glycosuria and insulin requirement was studied. During the

early stage of pregnancy the glycosuria was increased while during late pregnancy glycosuria and insulin requirements were decreased. A still greater reduction occurred during lactation. These reductions may better be accounted for by the passage of dextrose and dextrose-forming substances into the fetuses rather than to the passage of fetal insulin into the maternal circulation. The only indication of an actual improvement in the diabetic condition observed was the gain of maternal weight which occurred in 2 of the 3 pregnancies. Pancreatectomy performed near term in 7 pregnant dogs did not result in hyperglycemia in the presence, in utero, of 3 or more viable fetuses. Under similar conditions the administration of a small amount of insulin (3 units twice daily) to 6 dogs, did not suppress the antihyperglycemic effect which the products of conception near term exhibit; in fact, 4 out of 6 dogs were sensitive to insulin. All animals were autopsied for remnants of pancreas and other changes. The results show that a substantial difference exists between the metabolism of a pregnant dog, depancreatized near term and given little or no insulin and that of a dog depancreatized prior to pregnancy and maintained on a uniform diet and insulin. The latter experimental condition is more analogous to that which obtains clinically in the human patient. It would appear that the products of conception near term cause a decrease in hyperglycemia in some but not all dogs.—K. Brownell.

Mental deterioration associated with convulsions and hypoglycemia. Report of two cases. Darrow, D. C., Am. J. Dis. Child. **51:** 575. 1936.

During the past 7 years a number of specimens of blood from children entering the hospital during or soon after a convulsion were analyzed for blood sugar. Of these, 13 showed from 85 to 110 mg. per 100 cc. of sugar in blood; 3 from 110 to 125 mg. and 5 from 60 to 70 mg. Apparently hypoglycemia does not occur commonly in cases of convulsions in children. Two cases of convulsions in children with mental deficiency and roentgenologic evidence of anatomic changes in the brain are reported. Hypoglycemia occurred in both cases shortly after some convulsions but not after all. Hypoglycemia (sugar content 20 mg. per 100 cc.) could be produced by starvation in 1 case without inducing a convulsion. It is suggested that the disturbance in the regulation of blood sugar may be due to lesions in the brain. —M. B. G.

Diabetes mellitus and hypothyroidism with a report of a case. Durkin, J., M. Clin. North America **20:** 279. 1936.

The case reported occurred in a female of 38 in whom it was illustrated that by increasing the basal metabolic rate by the administration of thyroid substance the diabetes was aggravated. When the thyroid was discontinued and the basal metabolic rate was lowered the diabetes appeared less severe and was more easily controlled.—I. B.

Hyperglycemia and glycosuria. Correlation of insulin and epinephrin (Hyperglykämie und Glykosurie. Ein Beitrag zur Korrelation Insulin-Adrenalin). Edelmann, A., and G. Singer, Wien. Arch. f. inn. Med. **28:** 397. 1936.

From studies on the blood chemistry of diabetic and other patients and of rabbits it is concluded that the division of sugar between the plasma and the erythrocytes is dependent upon a delicate balance between the production of insulin and of epinephrin.—W. J. A.

The effect of various substances on the action of insulin. Fisher, A. M., and D. A. Scott, J. Pharmacol. & Exper. Therap. **58:** 93. 1936.

Spermine from fresh beef pancreas and basic substance from the thymus gland of beef were added to solutions of stock insulin and an insulin preparation containing zinc. The stock solution of insulin, 5.5 cc. was added to 1 cc. of water, buffer, spermine solution or thymus preparation. The effects of these substances on the hypoglycaemic action of insulin were determined on either rabbits or dogs. The results show that the addition of spermine to insulin solution containing zinc produced a sustained insulin action. The basic substance prepared from beef thymus glands also prolonged the hypoglycaemic action of insulin. The presence of zinc was essential for the sustained insulin action.—E. L.

Cholesterol content of blood in diabetic patients fed diets rich in fat. Freyberg, R. H., L. H. Newburgh and W. A. Murrill, Arch. Int. Med. **58:** 589. 1936.

In 21 patients with controlled diabetes, on diets rich in fat for from 2 months to 8 years, the cholesterol values of the blood during fasting were found to range between 126 and 223 mg. per 100 cc. The average was 176 mg. In the same number of normal control

subjects the values ranged between 130 and 186 mg. per 100 cc., with an average of 156 mg. The level of cholesterol in the blood of these diabetic patients could not be correlated with the amount of fat in the diets. Values only slightly higher than the highest for the control subjects were found in 8 of the 21 subjects. The significance of these higher values is not clear. Lack of control of the diabetes appears to be the major factor in the causation of hypercholesteremia in diabetic patients.—I. B.

Measurement of insulin action. Greeley, P. O., H. C. Berman, D. B. Tyler and D. R. Drury, Proc. Soc. Exper. Biol. & Med. **34:** 121. 1936.

In convalescent, depancreatectomized dogs, maintained with insulin, and then prepared for the experiment by withholding food and insulin for 24 hours, it was determined that about 0.01 U. of insulin per kilo per hour was required to maintain a constant, normal blood sugar. Amytal and nembutal both depressed the blood sugar of such animals receiving continuous injections of glucose and insulin.—M. H. F.

Carotenemia in diabetes. Heymann, W., J.A.M.A. **106:** 2050. 1936.

The author states that the blood serum carotene curves obtained in 10 diabetic children after the administration by mouth of carotene in oil were distinctly different from those obtained in 12 nondiabetic, healthy children and demonstrated that the metabolism of carotene is interfered with in diabetes. The carotene content of the blood, when it was once increased in the diabetic patients, failed to show the normal decline and remained elevated or even kept on increasing for from 10 to 14 days after the administration of the carotene in oil had been discontinued. The analogy with the hyperglycemic reaction after sugar is given by mouth to diabetic patients is striking and speaks in favor of assuming that the utilization of carotene has been interfered with in diabetes. The diabetic carotenemia can consequently no longer be explained merely by the high carotene content of the diabetic diet.—Abst., A.M.A. Service.

Hypoglycemia in the new born. Higgons, R. A., Am. J. Dis. Child. **50:** 162. 1935.

The author calls attention to the occurrence of convulsions in the newborn period which simulates those of cranial hemorrhage and advises that blood sugar determinations be made in all such conditions. He reports 2 cases which showed hypoglycemia and which improved upon the administration of maternal blood and hypodermoclysis of glucose. One of the mothers was a diabetic.—M. B. G.

Protamine insulin. Kerr, R. B., C. H. Best, W. R. Campbell and A. A. Fletcher, Canad. M.A.J. **34:** 400. 1936.

Depancreatized dogs on a liberal diet were kept sugar-free and in excellent condition with 1 dose of protamine insulin daily; an equivalent single dose of regular insulin is less effective in preventing glycosuria and more likely to provoke hypoglycaemic reactions. Addition of zinc salts to regular or protamine insulin also had the effect of prolonging the action. Protamine insulin has been used successfully in 25 human diabetics; it is valuable especially because the fall of blood sugar is less steep and reactions are rare.—D. L. T.

Some experiments with protamine insulinate. Lawrence, R. D., and N. Archer, Brit. Med. J., **1:** 747. 1936.

The authors confirm previous reports by Hagedorn, Root and their co-workers on the action of protamine insulinate in several cases of diabetes mellitus. This compound acts more slowly and for a longer period than ordinary insulin and a large dose can act for more than 2 days. It was found to be weaker in dealing with exogenous carbohydrate than regular insulin, but its action on endogenous sugar was nearly as good and rapid as that of ordinary insulin. The symptoms of hypoglycemia after protamine insulinate were less prominent than those due to ordinary insulin even at the same blood sugar level. A theory is presented to account for the activity of insulin and the role of the adrenal, thyroid and pituitary glands, in the regulation of sugar metabolism is discussed.—H. B. Friedgood.

Diabetes mellitus neonatorum. Report of a probable case. Lewis, E., and H. Eisenberg, Am. J. Dis. Child. **49:** 408. 1935.

A blood sugar content of 520 mg. per 100 cc. and a positive Benedict's test in the urine was found in an 11-day old colored child with an upper respiratory infection and pneumonia.

The child died in spite of insulin treatment and on post-mortem examination the pancreas showed an increase in the number of interacinar fibrous tissue and a reduction in the number of islands of Langerhans. The suprarenal glands were normal. The author considers this to have been a case of probable neonatal diabetes mellitus and states that White cites but 3 cases of congenital diabetes in which hyperglycemia was present at birth or shortly thereafter. The mother in the present case evidently did not have diabetes.—M. B. G.

Diabetes mellitus with extensive gangrene in early infancy. Limper, M. A., and A. L. Miller, Am. J. Dis. Child. **50:** 1216. 1935.

A case of diabetes mellitus with onset at the age of 9 days is presented. There was dry gangrene involving the left lower extremity to the midportion of the thigh. At autopsy a thrombosis was found involving the aorta extending into all its branches from the level of the left renal artery downward. The pancreas showed pancreatitis involving the head of the pancreas and acute degeneration of the islands of Langerhans in the body and tail. The mother of this infant had mumps at the time of delivery and the possibility of the pancreatitis being due to the virus of mumps is discussed. Four cases of gangrene associated with infantile diabetes are reported in the literature. There is a comprehensive discussion of diabetes with onset prior to the age of one year.—M. B. G.

Exercise in diabetes mellitus. Marble, A., and R. M. Smith, Arch. Int. Med. **58:** 577. 1936.

Exercise of short duration carried to the point of only mild fatague invariably caused a definite and often a marked increase in the concentration of sugar in the blood of young patients with severe or moderately severe diabetes who had not received food or insulin for several hours. The practical bearing of this finding on the use of exercise in the treatment of diabetes is discussed.—I. B.

Circulatory disturbances and certain electrocardiographic changes in diabetes mellitus (Zur Frage der Kreislaufstörungen und bestimmter electrokardiographischer Veranderungen beim Diabetes mellitus). Misske, B., and H. Schütt, Deutsches Arch. f. klin. Med. **178:** 359. 1936.

Among the circulatory disturbances observed in 522 patients suffering from diabetes mellitus, arteriosclerosis and hypertension were most common but acute cardiac insufficiency and hypotension occur in a large number of cases. Pneumonia caused death in these patients more frequently than either coma or circulatory disease. Electrocardiographic studies were made on 200 of these cases but only 35 were repeatedly observed. There was no relationship between the clinical symptoms of diabetes mellitus and the electrocardiographic changes. The authors deny that any specific change in the electrocardiogram is produced by diabetes mellitus. The many changes observed are due to the associated circulatory disturbances.—E. L.

Blood pressure responses to insulin. Pijoan, M., Proc. Soc. Exper. Biol. & Med. **34:** 37. 1936.

The intravenous injection of 15 U. of insulin in normal patients provoked a marked rise in blood pressure, with a significant increase in pulse pressure, which began about the time the blood sugar curve began its recovery toward the normal value from the hypoglycemic levels. This pressor response was more marked in patients with hypertension, and absent in 4 patients with Addison's disease. Diabetics reacted normally.—M. H. F.

Effects of betaine upon the cholesterol and bilirubin contents of blood plasma in diabetes mellitus. Rabinowitch, I. M., Canad. M.A.J. **34:** 637. 1936.

The administration of betaine hydrochloride (0.5 gm. t.i.d.) in 10 thoroughly-studied diabetics slightly lowered the plasma bilirubin and plasma cholesterol, but in the latter case the result was not statistically significant. There were signs of some improvement in carbohydrate tolerance.—D. L. T.

Clinical experiences with protamine-zinc-insulin and other mixtures of zinc and insulin in diabetes mellitus. Rabinowitch, I. M., J. S. Foster, A. F. Fowler and A. C. Corcoran, Canad. M. A. J. **35:** 239. 1936.

A buffered solution of protamine-insulin, to which was added 1 mg. zinc per 500 units, has been tested on a series of diabetic patients. A more prolonged lowering of blood sugar is obtained when zinc is added. In ten 'insulin-wasters' the average fasting blood-sugar during

8 weeks treatment with regular insulin, 46.5 units daily average, was 0.285%, as compared with 0.189 during a similar period of study of the same patients receiving 78.2 units protamine-insulin, and only 0.131% in a third period of treatment with 59.4 units protamine-zinc-insulin. The zinc appears actually to increase sensitivity to insulin, so that the new mixture cannot safely be given in such large doses as protamine-insulin; for the same reason, however, it is not contraindicated in coma and other emergencies. Crystalline insulin appears to exert a more prolonged action than regular insulin, and this may be due to its higher zinc content: spectrographic analysis shows that crystalline insulin may contain as much zinc as the prepared mixture above mentioned. In high concentrations (0.4%) zinc inhibits the action of insulin; in the indicated quantities, zinc is probably harmless and rapidly excreted.—D. L. T.

Insulin and the storage of live glycogen in anesthetized cats. Reid, C., J. Physiol. **87:** 121. 1936.

Fasting cats under chloralose anesthesia were given insulin by slow intravenous infusion. Blood glucose and liver glycogen samples were taken after initial, infusion and post-infusion periods. In both normal (11) and medulliadrenalectomized animals (9) liver glycogen increased during insulin infusion provided the initial blood glucose was high or sugar was injected. In both groups it fell shortly after insulin infusion was stopped. A post-insulin glucose rise did not occur in cats completely adrenalectomized. The protein sparing action of carbohydrate was followed by determining sulphate excretion in mg. per hour. In normal and adrenalectomized cats glucose infusion coincided with diminished sulphate excretion, but in depancreatized cats sulphate excretion diminished only after glucose plus insulin. The application of the results to the storage of liver glycogen is briefly discussed.—R. L. Z.

Daily variation of sugar content of blood and urine during treatment of diabetes mellitus. Rogers, F. L., Arch. Int. Med. **57:** 1027. 1936.

A study of 24 cases of diabetes mellitus showed a daily quantitative variation in sugar content of the blood and in the amount of sugar excreted in 16. In 9 cases the variation was slight; in 7 it was marked enough to interfere with satisfactory treatment when the injections of insulin were given before meals. The variation consisted in an early morning rise in the blood sugar, a parallel rise in excreted sugar and an afternoon fall in both. Meals did not seem to affect the curves. As a rule the variation was found in young patients with severe diabetes, while older patients with milder forms often failed to show it. The administration of insulin in anticipation of the early morning rise resulted in more satisfactory control than could be obtained otherwise, but there was no evidence that the total requirement of insulin could be reduced by this method of administration. A single dose of insulin given in anticipation of the peak failed to control the condition adequately throughout the 24 hours. One patient showing the variation was restudied after a year, and similar variability was found, indicating permanence.—I. B.

Ion distribution between plasma and erythrocyte in normals and hypochloremic diabetics (Ionverteilung zwischen Plasma und erythrocyten bei normalen und hypochloramischen Diabetikern). Schmitt, F., Arch. f. exper. Path. u. Pharmakol. **181:** 563. 1936.

By means of a specially constructed apparatus, described in the article, the author was able to separate plasma and erythrocytes with great exactness and study the Cl, Na, K and Ca content of each fraction in the normal person as well as in the diabetic. Arterial blood was used for the determinations. In diabetics the plasma contained less Na and more K and Ca than normal. Chloride was increased in the erythrocytes; the erythrocytes contained less of the cations and less water.—R. Cleveland.

Studies on insulin with protamine. Scott, D. A., and A. M. Fisher, J. Pharmacol. & Exper. Therap. **58:** 78. 1936.

Protamine solution 1 cc. (2.2 mg. protamine), prepared from salmon testes by Kossel's method, was added to 5.5 cc. of insulin solution. The hypoglycaemic effect of insulin with and without protamine was determined on groups of rabbits by injecting 2 U. of insulin per kg. and following the blood sugar for 12 hours. The hypoglycaemia, produced when insulin with protamine was injected, was twice as prolonged as when insulin alone was used. Protamine from the testes of the cohoe salmon, spring salmon, steelhead salmon and rainbow trout were equally effective. The addition of small amounts of zinc chloride to the protamine-insulin complex greatly sustained the hypoglycaemia.—E. L.